Universidade de Aveiro Departamento de Química
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Pedro Emanuel Pato Martins

Variabilidade da distribuição e das trocas de mercúrio entre a Ria de Aveiro e o Oceano Atlântico

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tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica da Doutora Maria Eduarda Pereira, Professora Auxiliar do Departamento de Química da Universidade de Aveiro.

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o júri

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palavras-chave

mercúrio; Ria de Aveiro; Oceano Atlântico; laguna; águas costeiras; balanços de massa; fluxos; transporte de contaminantes; ciclos de maré; partição; matéria suspensa particulada; seston; sedimentos.

resumo

A Ria de Aveiro (Portugal) é uma laguna mesotidal que comunica com o Oceano Atlântico através de um único e estreito canal. Durante quatro décadas, foram descarregados num braço da Ria efluentes contaminados com mercúrio provenientes de uma indústria de produção de cloro e soda caustica e estudos anteriores documentam que cerca de 33 toneladas de mercúrio foram retidos nos sedimentos da laguna.

O presente trabalho teve como objectivo aumentar o conhecimento dos processos de dispersão e sobre o comportamento do mercúrio durante o percurso estuarino (incluindo a importância do plâncton nestes processos), e a avaliação da extensão da transferência para o Oceano Atlântico. Tirando partido do privilégio da existência de uma única conexão com o Oceano, o canal de interface entre a Ria de Aveiro e o Oceano Atlântico serviu como um ponto ideal para a monitorização da variabilidade do transporte.

Para tal, foram recolhidas amostras de água no canal mencionado durante ciclos de maré com amplitudes de maré e descargas fluviais contrastantes. Para estudar os factores que controlam a partição do mercúrio, foi realizada uma caracterização físico-química da fracção dissolvida e dos sólidos suspensos finos e grosseiros. Relativamente à distribuição de mercúrio durante os ciclos de maré, as concentrações na fracção dissolvida, na matéria suspensa particulada e no seston variaram ao longo dos ciclos de maré e entre os ciclos de maré de diferente descarga fluvial e amplitude de maré. De um modo geral, e principalmente nas marés mortas, o mercúrio encontra-se associado maioritariamente à matéria suspensa particulada. As maiores concentrações foram observadas no Inverno, devido a maior erosão de sedimentos superficiais das zonas contaminadas por conjugação de maiores correntes e maior efeito de resuspensão induzido pelo do vento e pelas ondas. A composição, quer da matéria suspensa particulada, quer do seston variou ao longo dos ciclos de maré, com a diferente descarga fluvial e amplitude de maré, influenciando os níveis de mercúrio das partículas e a partição entre as fracções dissolvida e particuladas. Os resultados observados no canal são consistentes com a eficiente retenção de mercúrio na área contaminada.

Para avaliar a importância (e variação sazonal) do impacto da contribuição da Ria na distribuição do mercúrio na área costeira Atlântica, foram medidos os níveis de mercúrio em vários compartimentos da zona costeira adjacente: em sedimentos superficiais, em águas superficiais e profundas (incluindo na matéria suspensa particulada e na fracção dissolvida) e no biota. As concentrações de mercúrio dissolvido (reactivo e total) observadas em águas superficiais e profundas foram baixas. Os valores médios de mercúrio na matéria suspensa particulada variaram entre 0.2 e 0.6 μg g^{-1} e nos sedimentos entre 1 e 9 ng g^{-1}. Os organismos aquáticos apresentam níveis abaixo da legislação mas exibem alguma bioacumulação de mercúrio, com concentrações variando entre 0.05 e 0.8 μg g^{-1} (peso seco). Além disso, não se encontrou nenhum padrão sazonal nas determinações relacionadas com mercúrio. Os níveis encontrados na embocadura do estuário durante a vazante evidenciam a existência de transporte de mercúrio para a zona costeira. Apesar das elevadas concentrações de mercúrio encontradas em algumas zonas do interior da laguna, a vasta rede de ilhas e canais permite alguma dispersão e retenção dos contaminantes antes de estes atingirem as águas costeiras. Além disso, a baixa eficiência de retenção dos sedimentos marinhos locais contribui para a diluição do mercúrio transportado na matéria suspensa particulada numa área mais vasta, reduzindo o impacto na zona costeira adjacente.

A variabilidade das trocas do mercúrio dissolvido e particulado entre a Ria e o Oceano Atlântico sobre diferentes descargas fluviais e amplitudes de maré, foi estimada através de modelação numérica. Os fluxos de mercúrio foram estimados através do produto das concentrações de mercúrio de interesse pelos fluxos de água, os quais foram calculados por um modelo hidrodinâmico 2D de integração vertical. Os resultados demonstraram que comparativamente com o mercúrio total dissolvido e com o mercúrio total no seston, a fracção particulada tem um papel muito importante no seu transporte enquanto que a contribuição da fracção do seston foi sempre menor que 0.5%. Durante a maré viva de verão, cerca de 2% do mercúrio transportado no seston encontrava-se em formas orgânicas. O balanço de massa para as fracções de mercúrio revelou que a sua exportação para o Oceano Atlântico varia com o regime sazonal e das marés, principalmente em termos da importância relativa das fracções dissolvida e particulada. Esta variabilidade é particularmente importante no estabelecimento de estimativas de trocas de metais e na avaliação do risco ecológico associado ao impacto de transporte de mercúrio para o Oceano.

As trocas mais elevadas de mercúrio durante a enchente e vazante foram observadas na maré viva de Inverno como resultado de diversos factores que promoveram a remobilização de mercúrio na zona contaminada. Uma gama aproximada de valores de balanço de massa anual entre 42 e 77 kg mostra que a exportação de mercúrio dissolvido e particulado tem pouco impacto na zona costeira Atlântica adjacente à Ria de Aveiro, e que a recuperação da laguna da contaminação com mercúrio deverá ser um problema a longo prazo. A alternância de processos de erosão e deposição em zonas muito pouco profundas e a complexa geometria da laguna parecem limitar o transporte advectivo de mercúrio em direcção ao mar. Os tempos de residências na zona contaminada (cerca de duas semanas ou mais) parecem desempenhar um papel preponderante na retenção de mercúrio, ao dificultar a exportação para a zona central da laguna.

keywords

mercury; Ria de Aveiro; Atlantic Ocean; lagoon; coastal waters; exchanges; mass balance; fluxes; contaminant transport; tidal cycles; partitioning; suspended particulate matter; seston; sediments.

abstract

The Ria de Aveiro (Portugal) is a mesotidal lagoon that connects to the Atlantic Ocean by a single narrow opening. During four decades, a remote branch received a discharge of a mercury contaminated effluent from a chlor-alkali plant and previous studies documented that about 33 tonnes of mercury were retained in the sediments of the lagoon.

The present work is aimed at increasing the understanding of the processes of dispersion and of mercury behaviour during the estuarine transit (including the importance of plankton in these processes), and of evaluating the extent of transfer to the Atlantic Ocean. Taking advantage of the existence of a unique connection with the Ocean, the outlet channel between the Ria de Aveiro and the Atlantic Ocean served as an ideal monitoring point for the variability of the inputs.

Water samples were collected in the mentioned channel during tidal cycles with contrasting tidal ranges and river discharges. To study the factors controlling the mercury partitioning, a comprehensive physico-chemical characterization of the dissolved fraction and of fine and course suspended solids was performed. Considering the distribution of mercury during the tidal cycles, concentrations in the dissolved fraction, suspended particulate matter and seston varied during the tidal cycles and between the tidal cycles of different river discharge and tidal range. In general, and mainly in neap tides, most of the mercury is associated with suspended particulate matter. The higher concentrations were observed in winter as a result of the higher erosion of surface sediments in the contaminated areas by conjunction of wave and wind-driven resuspension and higher currents. The composition of both the suspended particulate matter and the seston varied during the tidal cycles, with the different river discharges and with tidal ranges, influencing the mercury content of particles and partitioning between dissolved and particulate phases. The results observed in the channel are consistent with an efficient retention of mercury in the contaminated area.

To assess the importance (and seasonal variation) of the impact of the Ria input on the mercury distribution in the Atlantic coastal area, total mercury levels were measured in several compartments of the adjacent coastal zone: in surface sediments, in surface and deep waters (including the dissolved phase and the particulate matter) and in biota. Dissolved (reactive and total) mercury concentrations, both at surface and at deep waters, were low. Mean mercury values in suspended particulate matter varied between 0.2 and 0.6 μg g^{-1} and in sediments between 1 and 9 ng g^{-1}. Aquatic organisms displayed levels below regulatory limits, but exhibited some bioaccumulation of mercury, with concentrations ranging from 0.05 to 0.8 μg g^{-1} (dry weight). No seasonal pattern was found in this study for mercury related determinations. Levels found in the estuary mouth during ebb tide provide evidence for the transport of mercury to the coastal zone. In spite of the high levels of mercury found inside some areas of the lagoon, the wide web of islands and channels allows some spreading and retention of contaminants before they reach the coastal waters. Moreover, the low efficiency of local marine sediments in trapping mercury contributes to a dilution of mercury transported in suspended particulate matter over a broader area, reducing the impact in the nearby marine coastal zone.

Numerical modelling was used to estimate the variability of dissolved and particulate mercury exchanges between the Ria de Aveiro and the Atlantic Ocean under different river discharges and tidal ranges. The mercury fluxes were estimated as the product of the appropriate mercury concentrations by the water fluxes calculated by a two-dimensional vertically integrated hydrodynamic model. Results showed that, compared to the total dissolved mercury and total mercury in seston, the particulate fraction plays an important role in the transport, while the contribution of the seston fraction to the transport of mercury was always lower than 0.5%. During spring tides, in summer, about 2% of mercury transported in seston was present in an organic form. The mass balance for the mercury fractions revealed that the mercury exports to the Atlantic Ocean varied with the season and tidal regime, mainly in terms of the relative importance of the dissolved and particulate fractions. This variability is particularly important for the establishment of long term metal budgets and to assess the ecological risks due to the local Oceanic budget of mercury.

The highest mercury exchanges during flood and ebb were observed in winter spring tide conditions as a result of several factors that promoted the resuspension of mercury in the contaminated area. An approximate range of values for the annual mass balance between 42 and 77 kg show that the export of dissolved and particulate mercury has little impact on the near shore region of the Atlantic Ocean and that the recovery of the lagoon from mercury contamination is likely to remain a long-term issue.
Alternating process of erosion and deposition associated with very shallow areas and the complex geometry of the lagoon seems to limit the advective seaward transport of mercury. The residence time in the contaminated area (about 2 weeks or more), appears to play a crucial role in the retention of mercury, by hindering the export to the main body of the lagoon.

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1. Introduction

Investigations in aquatic sciences play an important role in building a sustainable world, and the intense study of aquatic systems, like estuaries and coastal lagoons, has greatly improved the knowledge about the dynamic equilibrium of these precious systems, contributing to our understanding of the Planet as a whole. Nevertheless, there is still a tremendous need for more research in the field of aquatic sciences. The importance of coastal zones to the global ecological equilibrium has generated an increasing interest in the achievement and maintenance of the high quality status of these zones. In particular, coastal lagoons are among the most productive ecosystems, with a diversity of habitats that must be preserved. In fact, as an integral part of the life cycle of numerous fish species and other biota, these systems are of considerable ecological and economical value. However, the increase of great anthropogenic pressure from human settlements, agricultural wastes and direct discharges of sewage into the lagoons has given rise to severe pollution, jeopardizing the biodiversity and ecology of these incomparable areas.

Mercury is considered to be an environmental pollutant of the highest priority. As a result of the concern around mercury pressures and impacts, it is a priority hazardous pollutant under Europe Union regulations (including the Water Framework Directive; EC, 2000), under the OSPAR convention (Convention for the Protection of the Marine Environment of the North-East Atlantic) and environmental agencies across the world. Mercury and its compounds are highly toxic to humans, ecosystems and wildlife. First seen as an acute and local problem, mercury pollution is now also understood to be global, diffuse and chronic. Despite the recent setting of restrictions on anthropogenic emissions, mercury buried in sediments may be released to the water column through disturbances inducing resuspension or changes in the physicochemical environment (redox potential, temperature, oxygen, salinity), making it available to aquatic organisms (e.g. Rajar et al., 1997; Hung and Chmura, 2005) and also to transport. In addition, the transport of mercury from the sediment to the water column may also occur via the activities of burrowing organisms or by higher trophic level organisms feeding on benthic invertebrates (Mason et al., 2006). In coastal environments, the atmosphere is a major pathway for the transport of mercury between the land and the oceans, while riverine inputs, globally, are relatively small (Cossa et al., 1996; Fitzgerald and Mason, 1997). However, estuaries make a significant contribution to the mercury mass balance in local coastal environments (Laurier et al., 2003; Mason et al., 2006; Schäfer et al., 2006), particularly in estuarine plumes (Cossa and Gobeil, 2000). Estuarine systems are therefore very important transition zones for the understanding of the behaviour and fate of mercury, with potential implications for the global biogeochemical cycling of the metal.

The Ria de Aveiro (Portugal), a coastal lagoon that exchanges with the Atlantic Ocean, received, for almost 50 years, the effluent of a chlor-alkali industry, and consequently several tons of mercury were buried in the sediments of an inner basin. Most of the studies of mercury geochemistry in the Ria de Aveiro were focused in biota and sedimentary compartments of the most contaminated area. Little attention was given to the mercury behaviour during estuarine transit and to the study of the transport between the lagoon and the Atlantic Ocean. Considering the interest on the processes affecting the historical load of mercury, the study of the mercury transport was imperative to evaluate the future environmental health of the Ria and of the adjacent coastal marine area.

This thesis is organized in six chapters. In the first (and present), the motivations and objectives of the work are described, according to the problematic of the study site. In the second chapter, named “Mercury in aquatic systems” a description of mercury behaviour in aquatic systems is presented, with special emphasis on the importance of mercury cycling.

In the third chapter, the analytical methods used in the study (from sampling to data processing) are described and quality control information given.

Chapter four describes and discusses the results of studies of the Ria inputs in the channel. In the fifth chapter, the state of the Atlantic coastal zone is described and discussed. In chapter six, the results of the numerical simulation of the estuarine transport are presented and discussed, including implications of the mass balance results. In chapter seven, some overall considerations are discussed.

1.1 Ria de Aveiro: problematic of mercury contamination

The Ria de Aveiro (Figure 1.1) is a coastal lagoon located on the north-western coast of Portugal (40° 38’N, 8° 45’W), that connects to the Atlantic Ocean by a single narrow opening (1.3 km long, 350 m wide and 20 m deep; Figure 1.1, C). The tides are predominantly semidiurnal with the tidal range varying from 0.6 m in neap tides to 3.2 m in spring tides, with an average of about 2 m (Dias et al., 2000), being defined as a meso-tidal coastal lagoon. The deepest areas of the lagoon are confined to the outlet channel and to small areas close to the lagoon mouth, at the western boundary of the lagoon, where the depths may reach values of the order of 20 m. Elsewhere, at the inner parts of the lagoon, the depths are, in general, lower than 3 m, and more frequently close to 1 m, namely at the upper reaches of the lagoon. In the main navigation channels, dredging operations maintain a depth of approximately 7 m.

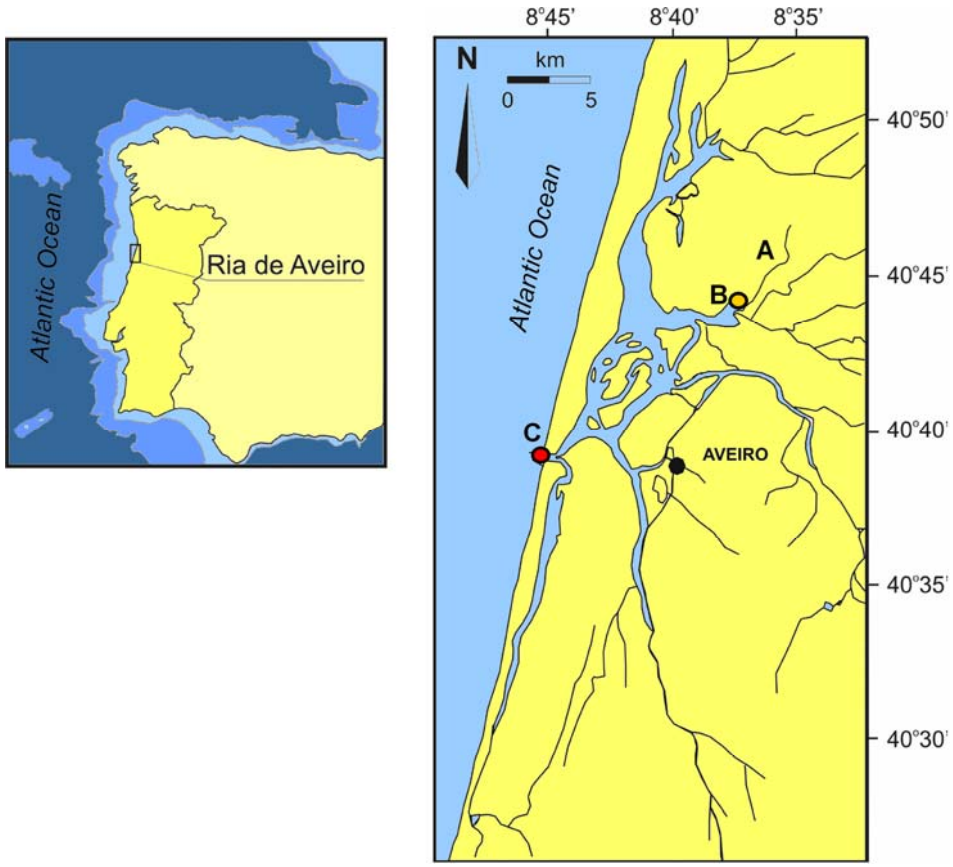


Figure 1.1 - Map of the Ria de Aveiro (A, Estarreja channel; B, Laranjo Basin; C, Outlet channel)

The lagoon is 45 km long with a maximum width of 10 km, covering an area of approximately 83 km^2 of wetland at high tide (spring tide) and 66 km^2 at low tide. It receives water draining from a catchment area of almost 3600 km^2, with several rivers carrying an average of 1.8 $\times 10^6$ m^3 freshwater into the lagoon during a tidal cycle (Dias et al., 2000). The Rio Vouga, with a drainage area of 2425 km^2 (Dias et al., 2000), is the main river discharging into the Ria de Aveiro. The average flow of the Rio Vouga, 25 m^3 s^{-1}, corresponds to 60% of the freshwater discharged into the lagoon. Concerning the connection with the sea, the lagoon exchanges a mean volume of water of 89 × 10^6 m^3 in tides ranging from 1 to 3 m. The estimated lagoon tidal prism (here defined as the volumetric flux passing across one section in a flooding cycle) is 136.7 Mm^3 for maximum spring tide and 34.9 Mm^3 for minimum neap tide (Dias et al., 2000). The total mean river discharge into the lagoon during a tidal cycle is comparatively small (about 1.8 Mm^3) and, therefore, semidiurnal tides are the major factor influencing the hydrodynamics of the lagoon (Dias et al., 2000) due to the low freshwater input compared with the tidally driven circulation. Extreme conditions of strong wind may induce particular circulation patterns, mainly in shallow

areas and wide channels (Dias et al., 2003). For more detailed information, please see Dias et al. (2000; 2003).

As a consequence of the combined effects of the freshwater discharge (from the tributaries and diffuse runoff) and tidal penetration, the Ria de Aveiro exhibits a longitudinal gradient of salinity from about less than 0.5 in the rivers, to about 36 at the bar entrance. The Ria has a complex and irregular topography, with three main channels irradiating and with successive ramifications from the mouth, forming a multiestuarine ecosystem. Secondary branches originate from the three main channels to form a tight net of channels, islands and mudflats.

The growth and development of industry and agriculture has caused pollution problems with inputs from wastewaters and runoffs from several anthropogenic activities (e.g. agricultural activities, the chemical complex of Estarreja, fish farming and nautical activities) which have adversely affected the water quality. For more than four decades, a chlor-alkali industry discharged an effluent rich in mercury in a remote branch (Estarreja channel, Figure 1.1, A) of the lagoon that ends in an inner bay of 1.5 km^2, Laranjo Basin (Figure 1.1, B). Most of the discharged mercury settled in the Laranjo Basin, with the highest mercury concentrations in sediments (maximum of 35 $\mu g\ g^{-1}$) buried at 30 - 40cm depth (Pereira et al., 1998a), corresponding to the period of maximum industrial production. The accumulation of mercury in the lagoon was estimated as 33 t of mercury, much of which (about 27 t) is known to be sediment-associated in the Estarreja channel and in the Laranjo Basin (Pereira et al., 1998a). In the past decade, the industry changed its technology and the discharge of anthropogenic mercury diminished considerably (Pereira, 1996). The highest concentrations of Hg in surface sediments are found in the upstream channel where the effluent was discharged during decades, with concentrations of 50 $\mu g\ g^{-1}$ (Coelho et al., 2005), and values up to 300 $\mu g\ g^{-1}$ (Pereira et al., 1998a). In the Laranjo Basin, levels in surface sediments range between 0.22 and 23 $\mu g\ g^{-1}$ (Ramalhosa, 2002; Coelho et al., 2005). These exceptionally high concentrations make the Ria a hotspot in terms of mercury contamination on the southwest Atlantic coast of Europe (OSPAR, 2000).

1.2 Motivations of the study and objectives

Hitherto studies in the Ria system have focused almost exclusively on the mercury biogeochemistry, mainly in terms of the sedimentary cycling in the most contaminated area (Pereira et al., 1998b; Ramalhosa et al., 2001; Ramalhosa et al., 2006a) and effects in biota (Abreu et al., 2000; Pato et al., 2002; Coelho et al., 2005; Coelho et al., 2006; Pereira et al., 2006; Coelho et al., 2007; Válega et al., in press). The first studies carried out in this lagoon estuarine system

(reviewed by Pereira (1996) and Ramalhosa (2002)) revealed the mercury contamination of the Ria and identified the chlor-alkali discharges as the source of contamination (Hall et al., 1987). As a result of concerns over the high levels observed in the sediments of the Estarreja channel and the Laranjo Basin, some works studied the potential of mercury remobilization from sediments of the Laranjo Basin. Estimated diffusive fluxes through the sediment-water interface indicate that the exported quantity by diffusion from interstitial waters is small and fail to explain the levels present in the water column (Ramalhosa et al., 2001; Ramalhosa et al., 2006a; Ramalhosa et al., 2006b). Much of the mercury present in the surface sediments of this basin is associated with organic matter and iron oxyhydroxides (Ramalhosa et al., 2006b), but tidal dynamics have been identified as a potential forcing for the mobilization and dispersion of particulate mercury escaping when the surface sediments are resuspended. This mercury may be transported by the tide to the rest of the lagoon (Pereira et al., 1998b; Ramalhosa et al., 2001; Monterroso et al., 2003) and ultimately may reach the Atlantic Ocean through its single outlet. In addition, the biogenic component of seston can play an important role in the transport due to the bioaccumulation of mercury in the contaminated areas and its tidal mobility (Monterroso et al., 2003; Ramalhosa et al., 2006a).

Given the knowledge of mercury behaviour in the Ria de Aveiro obtained in previous studies and, together with a solid review of published work in other systems around the world, the present work is aimed at furthering those studies, and increasing the understanding of processes of dispersion and bioaccumulation of mercury, taking into account the importance of plankton in these processes. Of special interest is the assessment of mercury behaviour during the estuarine transit and the evaluation of the extent of transfer to the Atlantic Ocean through its single outlet. The European Water Framework Directive offers an unparalleled opportunity for the improvement of the ecological quality of the aquatic systems, but is unquestionable that the success of its implementation depends on the knowledge of the dynamic of pollutants in transitional waters. These studies of the mercury cycling in the lagoon water-column and of the mercury exchanges with the Ocean are very important, not only to understand the processes regulating the distribution within the system, but also to find responses to the contamination and to the impact in the regional mercury cycle.

In brief, the main (still unanswered) questions that this research approaches are:

- What are the factors controlling the distribution of mercury species between the dissolved pahse and the suspended particulate matter in the communication between the lagoon and the Ocean?

- Does the amount of mercury transported from the more contaminated areas contribute to increase significantly the mercury levels in the Ria and in the adjacent coastal zone?
- Is mercury retained in the sediments of the adjacent coastal zone?
- Are organisms of the adjacent coastal zone bioaccumulating mercury in levels of concern?
- Are variations in the tidal range and river discharge important to the mercury transport?
- Does plankton play an important role in the dispersion and transport of mercury?
- Is mercury transported in toxic forms?

In order to answer these questions and the underlying hypotheses, the following steps were taken:

- Water samples were collected in the channel of communication between the Ria de Aveiro and the Atlantic Ocean during tidal cycles with contrasting tidal ranges and river discharges. Taking advantage of the existence of a unique connection with the Ocean, the channel serves as an ideal monitoring point for the variability of the inputs. To study the factors controlling the mercury partition, a comprehensive physico-chemical characterization of the dissolved phase and of the fine and course suspended solids was carried out.
- The marine coastal zone under the influence of the lagoon was investigated in several field studies in different seasons. The mercury distribution between the water column, the sediments and the biota were evaluated.
- In the absence of continuous measurements of exchanges of water between the Ria de Aveiro and the Ocean, the study of transport had to rely on tools that supply that information. Hence, a hydrodynamic model was used to estimate the exchanges between the Ria and the Ocean.

Parts of this work have been published as:

- Inputs from a Mercury-Contaminated Lagoon: Impact on the Nearshore Waters of the Atlantic Ocean. Pato P, Válega M, Pereira E, Vale C, Duarte A C, Journal of Coastal Research 24, *in press*.

- Mercury fluxes between an impacted coastal lagoon and the Atlantic Ocean. Pato P, Lopes C, Válega M, Lillebø AI, Dias JM, Pereira E, Duarte AC, 2007. Estuarine, Coastal and Shelf Science, *in press*. http://dx.doi.org/10.1016/j.ecss.2007.08.010

2. Mercury in aquatic systems

Mercury in the aquatic environment

Mercury stands in an unique (although infamous) position in the history of environmental contamination, since it was the first contaminant for which a direct relation among low concentrations in an aquatic system, a bioaccumulation along a trophic chain, and an important impact in human health, was proven (Kudo et al., 2000; Hamada and Osame, 1996). Moreover, it is one of the few metallic contaminants responsible for human fatalities due to the ingestion of contaminated food.

The concern with contamination of aquatic systems arises as they represent unique habitats to a vast number of species, being an important support for commercial fishery and recreation activities. Coastal ecosystems very often support large urban and industrial areas, and are, as a result, significantly affected by human activities.

In aquatic systems, mercury is found mainly as inorganic salts and as organomercuric species, compounds with a covalent bond between the metal and carbon atoms (C-Hg). The compounds most likely to be found under environmental conditions are the mercuric salts ($HgCl_2$, $Hg(OH)_2$ and HgS), methylmercury compounds like CH_3HgCl (methylmercury chloride) and CH_3HgOH (methylmercury hydroxide) and, in small amounts, other organomercurics like $(CH_3)_2Hg$ (dimethylmercury) and $C_6H_5Hg^+$ (phenylmercury) (USEPA, 1997). It is important to note that it can also be found in associations between inorganic species of mercury and organic material, however without the formation of covalent bonds, which are not classified as organomercuric species (USEPA, 1997). In addition to other categorizations, the more important mercury species in the environment can also be arranged according to their behaviour (Farago, 2000):

- Volatile species: Hg^0, $(CH_3)_2Hg$.

- Soluble/associable to particles reactive species: Hg^{2+}; HgX_2; HgX_3^-, HgX_4^{2-} (where $X=OH^-$, Cl^- ou Br^-); HgO in aerosols; Hg^{2+} complexed with humic matter.

- Non-reactive species: CH_3Hg^{2+}, CH_3HgCl, CH_3OH; $Hg(CN)_2$; HgS and Hg^{2+} associated to sulphur in humic matter fragments.

2.1 Main sources and applications of mercury

The emissions of mercury to the environment can be grouped in four categories (Pacyna et al., 2001; Wang et al., 2004):

- Natural emissions by mobilization of mercury occurring naturally in the earth crust (weathering), through volcanic activity, erosion of rocks and volatilization from the surface of oceans;

- Anthropogenic emissions associated with mobilization of impurities in materials such as fossil fuels – particularly coal and, to a lesser extent, gas and petroleum – and extracted, treated and recycled minerals;

- Anthropogenic emissions as a result of mercury used in products and mobilized in production processes. These emissions can occur either during the production, or through spills, discharges or waste incineration;

- Remobilization of mercury resulting from past anthropogenic emissions which were buried in soils, sediments and water masses.

Therefore, anthropogenic activities are the main source of this metal into the environment. Several point emissions are identified as more important, mainly chlor-alkali production, mining activities, smelting of metal ores enriched in mercury, coal and oil combustion, cement production, waste incineration (medical and municipal) and lightning industry.

The growth of mercury use in the XX century followed the scientific development, exceeding 3000 applications. However, the knowledge of its toxicity and persistence in the environment led to the search for substitutes, which originated a drop in its use in some sectors (Simon et al., 1998). Among the main mercury uses, special emphasis is usually given to the chlor-alkali industry and electric devices industries. The chlor-alkali industry, where the mercury is used as a cathode in the electrolysis of brines, is the main consumer (WHO, 1991). According to the WHO (1991), a considerable amount of mercury is used in the extraction of gold, an activity responsible for the contamination of several aquatic systems (Limbong et al., 2003; Wang et al., 2004).

The estimation that in the last 150 years mercury emissions have tripled (Mason et al., 1994), demonstrates the anthropogenic influence in the mercury global cycle. This study also reports that 70-80% of the current emissions of mercury to the atmosphere are of an anthropogenic origin. According to Lucotte et al. (1999), the increase in anthropogenic activities during the XX century can be responsible for a 2 to 3 fold increase in the rate of mercury deposition in several lakes in Canada. In Europe, the anthropogenic emissions of mercury into the atmosphere in 1995 were estimated at 342 ton (Pacyna et al., 2001). More than half of that is believed to come from coal burning in power plants and household heating, and the chlor-alkali industry is responsible for about 12% of these emissions. According to the same study, Portugal contributed with only 0.4%, Russia being the country with the highest emissions into the atmosphere with more than

25%. The 1.5 ton of mercury estimated for the emissions by Portugal include coal burning in thermoelectric power plants (0.20 ton), cement production (0.70 ton), production of sodium hydroxide (0.5 ton) and other diverse activities (0.1 ton).

The comparison of emissions for 1995 with the estimates of emissions for 1982, 1987 and 1990 (Pacyna el al., 2001) shows decreases and increases in some sectors, justified by the variability of the corresponding activities or by improvements in the methods of evaluation, which in the first studies resulted in sub estimations. This was the explanation presented for the increase reported in chlor-alkali industry emissions from 71 ton in 1982 to 235 ton in 1987.

2.2 Mercury cycling in aquatic systems

Due to its influence in important processes, the biogeochemical cycle of mercury is one of the most interesting and complex elemental cycles (Fitzgerald and Mason, 1997; Mason and Sheu, 2002). Mercury is particularly reactive in the environment, shifting rapidly between the four interconnected compartments (atmospheric, terrestrial, aquatic and biotic). Some species stand out due to their importance in the global behaviour. The atmospheric behaviour is dominated by the presence of elemental gaseous mercury, whereas the divalent inorganic mercury controls the fluxes to the aquatic and terrestrial compartments. In the soils, the Hg^{2+} associated with soil organic matter plays an important role. In the aquatic compartment, the main role is related to the pair Hg^{2+}-ligands in the water and Hg^{2+} associated with the sediment, while in the biota, methylmercury display an unquestionable importance (Wiener et al., 2003). Figure 2.1 represents the biogeochemical cycle of mercury, which although simplified, intends to highlight the importance of routes and processes that influence the exposure of biota to methylmercury.

Natural waters are usually Hg^0 (aq) saturated, when compared with the atmosphere above, promoting thereby a flux from the water to the atmosphere (Morel et al., 1998; Gårdfeldt et al., 2001; Guentzel and Tsukamoto, 2001). This volatilization of elemental mercury results in a decrease in its half-life in the aquatic compartment and is attributed to several processes: demethylation, bacterial reduction in the water and sediment, reduction of Hg(I) and Hg^{2+} by humic and fulvic acids, physiologic reduction by phytoplankton (in an elimination route) and photochemical reduction of Hg^{2+} to Hg^0 (Morel et al., 1998; Costa and Liss, 1999; Fitzgerald et al., 2007).

Atmospheric mercury exists mainly in the form of elemental mercury vapour (90 to 99%), particle-bound mercury (<5%) and gaseous divalent mercury (e.g. $HgCl_2$) (<5%). The atmospheric

mercury can be oxidized to mercuric, Hg^{2+}, a transformation taking place mainly at the solid-liquid interface of fog drops and clouds (Morel et al., 1998). Once oxidized, and although only 30% of the planet surface is covered with land, 60% of atmospheric mercury is deposited on land and 40% in the water (Mason et al. 1994). To this apparent contradiction it is proposed a contribution of the oxidation and deposition of metal in aerosols, more abundant over the continents. Nevertheless, the return of mercury from the atmosphere to the terrestrial surface occurs essentially through wet deposition of dissolved Hg^{2+}. The residence time of Hg^0 in the atmosphere is about 1 year, long enough to allow a distribution along the planet before returning to land, lakes, seas and frozen areas. For this reason, and although the mercury sources considered as most important in environmental terms are those from anthropogenic origin associated to industrialized zones, which display more local impact, the impact of mercury is absolutely global, affecting even remote areas of the planet.

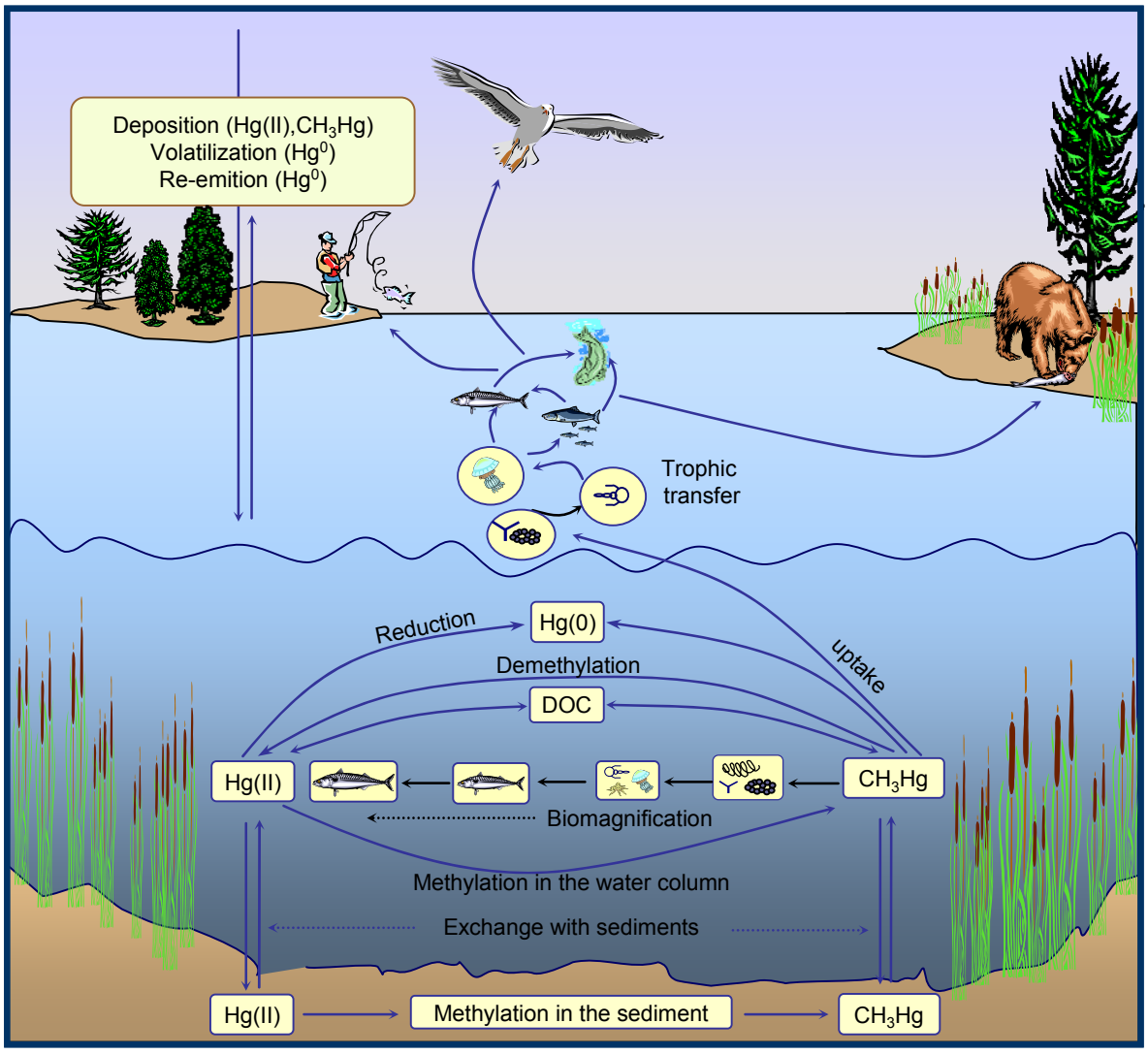


Figure 2.1 - Simplified cycling of Hg in aquatic systems (adapted from Wiener et al., 2003).

In oceanic waters, after undergoing a complex set of chemical and biological transformations, a portion of the Hg^{2+} is reduced to Hg^0 and returns to the atmosphere and another portion is incorporated in the sediments (Mason et al., 1994). The sedimentary compartment therefore constitutes an important destiny and reservoir of mercury in aquatic systems (Pereira, 1996; Lucotte et al., 1999; Kim et al., 2004; Hung and Chmura, 2005; De Marco et al., 2006). In fact, when polluted freshwaters reaches an estuary the dissolved mercury can flocculate and the slow down of the flow induces the settling of contaminated particles.

Disturbances of contaminated sediments by e.g., dredging, may lead to mobilization of Hg deposited in deep sediments. The contact of anoxic sediments with the oxygenated water column results in the mobilization of mercury associated to sulphides in the sediments (Calmano et al., 1996; Petersen et al., 1997). The changes in mercury species resulting from sediments resuspension can also include methylmercury production (Bloom and Lasorsa, 1999).

The methylation occurring preferentially in the sediments is a fundamental route in the bioaccumulation of mercury, since it results in the formation of the most rapidly accumulated specie in the biota, resistant to environmental transformation processes.

2.3 Major mercury associations in aquatic systems

For a better understanding of mercury behaviour in aquatic systems, it is essential to identify the predominant forms of the metal in compartments such as the water column, the sediments and the biota. In the water column, it is important to differentiate the dissolved fraction from the particulate fraction. The former is defined operationally as the fraction constituted by all the forms of the metal that are not retained by a 0.45 μm pore size filter. In opposition, the suspended particulate matter (SPM) is defined as the material retained by the same filter. Being an operational definition, it is important to keep in mind that a dissolved fraction established in this way will contain particles, e.g. colloids, which occur naturally and comprise dimensions below the referred pore size. Stordal et al. (1996) observed relevant amounts (57±20%) of mercury in the dissolved fraction associated to colloidal material of several estuaries. Guentzel et al. (1996) reported even higher quantities (79-87%) of dissolved mercury associated to the colloidal fraction in estuarine samples of low salinity (0-4), this fraction decreasing to 40-50% in zones of higher salinity, corroborating the influence of salinity in mercury partition. According to these two studies, the colloidal organic carbon plays an important role in the partitioning. Moreover, the distribution between dissolved, particulate and colloidal fractions shows spatial and seasonal

variability and also with water column depth (Morel et al., 1998). This partitioning of mercury in the water column, for example in coastal lagoons and estuaries, has a very important environmental impact, since it controls not only the export of metal to the coastal zone, but also the ability of these systems to accumulate large amounts of mercury in their sediments (Lacerda and Gonçalves, 2001).

The dissolved fraction can contain mercury in its soluble inorganic forms (just as $HgCl_2$, $Hg(OH)_2$, $Hg(HS)_2$, Hg^0, HgS_2^{2-} e $HgCl_4^{2-}$, depending on the conditions of the aquatic medium), complexed with organic ligands (e.g., humic substances) and also as organic species, such as CH_3HgCl, CH_3HgOH, CH_3Hg-humic substances (Ramalhosa, 2002). The presence of chloride and the pH exert an influence on the most important dissolved mercury species (Pereira, 1996; Ramalhosa, 2002). In fact, and according to thermodynamic calculations, in surface waters, the divalent mercury is not found as free Hg^{2+}, appearing instead complexed with variable amounts of hydroxide and chloride (Figure 2.2). In waters with low pH and low chloride content, the dominant species is the Hg^{2+} ion, while for higher pH, the Hg^{2+} ion undergoes rapidly hydrolysis, originating ($Hg(OH)_2$, $Hg(OH)_3^-$, $Hg(OH)^+$). At high chloride concentrations the content of chloro-complexes ($HgCl^+$, $HgCl_2$, $HgCl^3_-$, $HgCl_4^{2-}$) increases (Pereira, 1996). Mercury chemistry is not only influenced by the pH and chloride content, but also by other factors, such as the presence of organic ligands (with special reference to humic and fulvic substances), the oxygenation and the level of organic matter in water (Pereira, 1996; Benoit et al., 2001; Ravichandran, 2004).

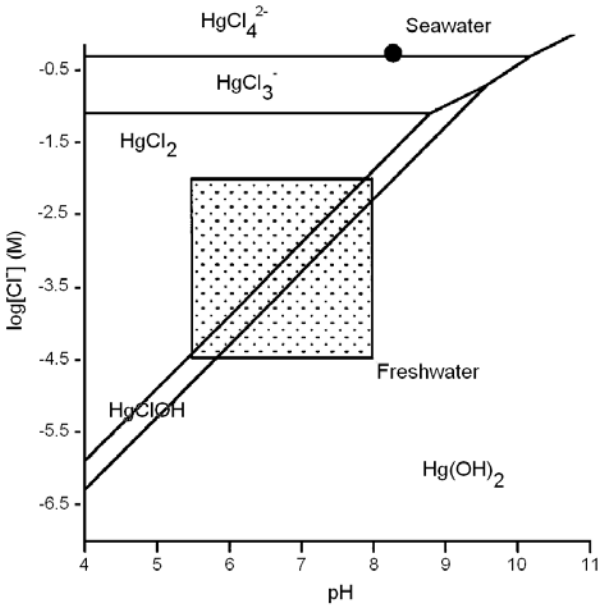


Figure 2.2 – Dominance diagram of hydroxo- and chloro-complexes of Hg^{2+} as a function of pH and chloride concentrations (Morel et al., 1998).

Depending on their stability, mercury species in the dissolved fraction can be classified as reactive or non-reactive. The reactive mercury species include inorganic dissolved species, elemental dissolved mercury and labiles mercury complexes, while the non-reactive species are the mercury species that bind through covalent bonds to carbon atoms and non-labile mercury complexes with organic matter (Válega, 2002; Ramalhosa, 2002; Fitzgerald et al., 2007). The reactive mercury fraction is also known as easily reducible mercury and is operationally defined as the mercury measured in a reduction step with stannous chloride.

In estuaries, the behaviour of mercury in the dissolved fraction depends upon several processes taking place in the mixing of two distinct water masses – fluvial and maritime. The metal concentrations can show conservative or non-conservative behaviour, meaning that the distribution of dissolved species is not always a function of the simple dilution of freshwater with saltwater. The reactivity of the metal in a particular system can thereby be characterized, allowing the evaluation of the risk of transport to adjacent coastal zones. A non-conservative behaviour of mercury in estuaries is frequently observed (Guentzel et al., 1996; Coquery et al., 1997; Benoit et al., 1998; Laurier et al., 2003), with a major role attributed to the removal of metal from the water column by organic matter (Benoit et al., 1998) and colloidal matter (Guentzel et al., 1996).

In the particulate fraction, precipitates can be found containing mercury (HgS), mercury species formed by adsorption and co-precipitation (HgS, $Hg(OH)_2$, $HgCl_2$) and adsorbed to organic matter (Morel et al., 1998; Válega, 2002; Laurier et al., 2003). Due to the ability of bioaccumulation of some mercury species, this fraction also includes the compounds that are able to enter in cells or simply adsorb at the surface of organisms (Ramalhosa, 2002). The organic content of the particles seems to influence the association of mercury with the particulate fraction (Mason and Sullivan, 1998), with an important role attributed to the humic fraction (Jingsheng et al., 1995).

In the water column, mercury is preferentially associated to suspended particulate matter (Coquery et al., 1997; Cossa et al., 2001; Le Roux et al., 2001; Laurier et al., 2003; Schäfer et al., 2006; Fitzgerald et al., 2007). Once the particles usually have negative charge at the surface, the adsorption of cations is favourable comparatively with the adsorption of anions, becoming a key process of mercury transport in this compartment (Pereira, 1996; Ramalhosa, 2002). In fact, and although mercury is thermodynamically characterized by stable dissolved complexes (either through association with humic matter in freshwater, or through complexation with chloride in saltwater), the dissolved forms are not the more abundant (Pereira, 1996; Le Roux et al., 2001). With respect to the affinity with the particulate matter, according to Diego et al. (2001), the

association of Hg^{2+} ion with particulate matter is stronger than for $MeHg^{+}$. According with the same study, in spite of the fact that the formation of the chloro-complexes (of Hg^{2+} and also of $MeHg^{2+}$) favours the desorption of the metal from the particles, a large percentage of Hg^{2+} could settle down towards the surface sediments of estuaries inner zones, after flocculation of suspended matter due to salinity increase.

The associations that mercury can establish in an estuary will depend on the complex relation between several physical and geochemical processes, like the mixture of fluvial and marine suspend matter, the flocculation of colloidal material, the adsorption and desorption in locations of different salinity, the production of organic matter by phytoplankton, the mobilization of iron and manganese in reducing sediments and the resuspension of sediments (Hatje et al., 2001; Le Roux et al., 2001).

Concerning the sedimentary compartment, after deposition of suspended particulate matter in the sediments, the mercury species undergo various chemical reactions, depending on the physico-chemical conditions of sediments, such as pH and redox potential, which control the adsorption and retention processes of mercury. In addition, the retention of mercury in the sediments is also controlled by the presence of iron and manganese oxides and oxyhydroxides, sulphides and organic matter (Chapman et al., 1998). As redox potential decreases, mercury precipitates as sulphides, due to its low solubility. In very reducing sedimentary environments, mercury can be found in the elemental form. In oxic conditions, the sulphides are oxidized to sulphates and the Hg^{2+} ion can associate with iron and manganese oxides and oxyhydroxides (Ramalhosa, 2002). The reduction of these oxides and oxyhydroxides leads to the remobilization of mercury from the sediments to the porewaters.

It is nowadays recognized that the presence or absence of some mercury species are the main factors controlling the behaviour and fate of the metal in aquatic systems and that different species present different toxic properties. The Figure 2.3 displays a diagram with some important routes of transformation of different mercury species in the aquatic environment.

Of all the mercury compounds, methylmercury has a special interest, not only for its high toxicity, but also because it accumulates along the trophic chains. Two of the methylmercury forms, the methylmercury hydroxide (CH_3HgOH) and the methylmercury chloride (CH_3HgCl), occur both in freshwater and saltwater, being the former dominant at low chloride concentrations and the latter prevailing at high chloride concentrations. Dimethylmercury volatilizes easily of the water surface and is generally not persistent in aquatic systems in significant concentrations. Only in deep waters (because of its long residence times far from the surface) it is found in detectable

levels. Dimethylmercury is unstable and decomposes in monomethylmercury which can be an important source of the former (Fitzgerald and Mason, 1997). Keeping this in mind, and unless where explicitly mentioned, the references to methylmercury regards to monomethylmercury.

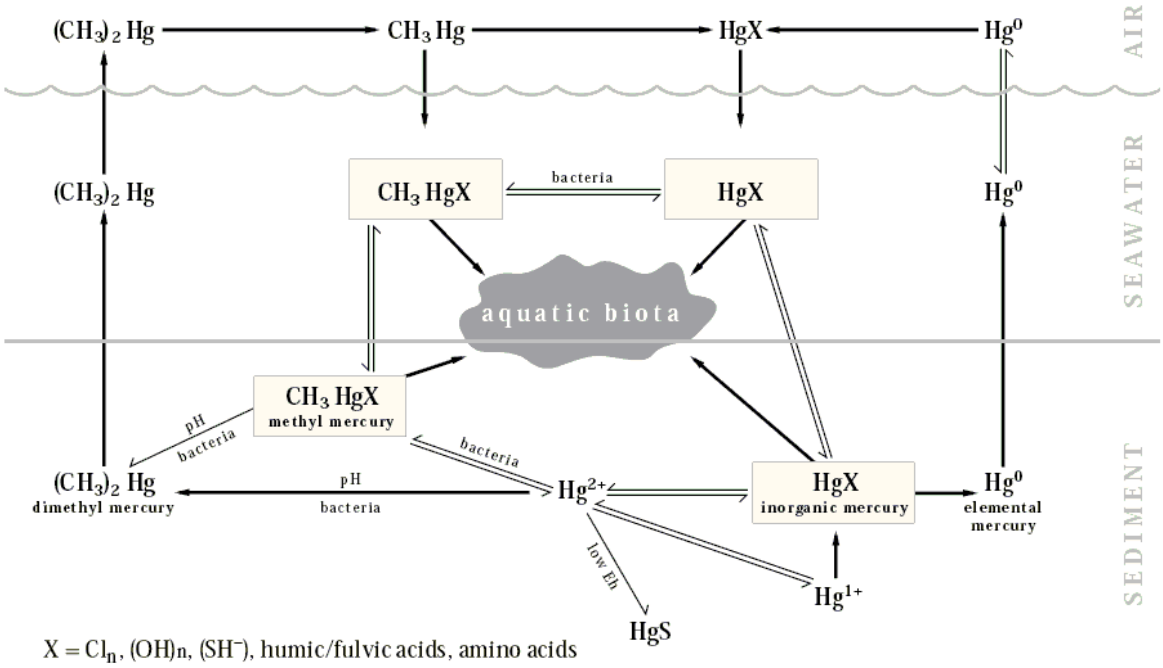


Figure 2.3 - Important pathways of mercury speciation in the aquatic environment (Beckvar et al., 1996).

2.3.1 Particle-water interactions – distribution coefficients

During transit through the estuarine mixing zone, chemical gradients are expected to modify to varying degrees the particles and the dissolved species. In such systems, the partitioning of chemicals is without question of particular interest since the reaction-controlling variables are subject to rapid and significant change. On one hand, particle composition can be modified by biogeochemical reactivity occurring both in the sediment (including through interactions/exchanges between sediment and the overlying water) and in the water column during suspended particle transport (Turner and Millward, 2000; Laurier et al., 2003; Turner et al., 2004). One the other hand, shifts in the equilibrium of dissolved species are very prone to occur concomitantly to changes in the dynamic composition of estuarine water. To conclude, interactions between both phases are also instrumental to establish the relative concentrations of a chemical in each phase. Hence, the resulting distribution of the chemical between suspended particles and water determines its reactivity, bioavailability, transport and fate.

The relative affinity of trace metals for dissolved and particulate phases in aquatic systems can be evaluated by the distribution coefficient, K_D:

$$K_D = \frac{C_{part}}{C_{diss}}$$

where C_{part} is the total particulate Hg content (ng kg^{-1}) and C_{diss} is the total dissolved Hg concentration (ng L^{-1}). High values of K_D suggest a strong affinity for the particulate phase.

The partitioning between these two phases is dependent on estuarine controlling variables, including salinity, pH, availability of complexing species and the physical and chemical characteristics of suspended particles, including its concentration. Many investigations have been devoted to assessing the influence of such factors in the particle-water interactions and in the resulting distribution between phases (Turner, 1996; Millward and Glegg, 1997; Benoit and Rozan, 1999; Lofts and Tipping, 2000; Lawson et al., 2001; Le Roux et al., 2001; Choe et al., 2003; Laurier et al., 2003; Millward and Liu, 2003; Turner, 2003; Turner et al., 2004).

2.4 Mercury methylation in aquatic systems

Factors that promote and inhibit methylation affect the distribution of inorganic and inorganic mercury, this process deserving special attention. The biotic and abiotic methylations occur in several conditions, but in aquatic systems the biomethylation is the predominant route, being admitted that the methylation by bacteria is a response of this organisms to the toxicity of mercury (Baldi, 1997; Guentzel and Tsukamoto, 2001). The sulphate-reducing bacteria (anaerobic) are the primary methylators of mercury in both lacustrine and estuarine sediments (Beckvar et al., 1996; Wiener et al., 2003). Methylmercury production also occurs at the sediment-water interface in well-mixed sediments, potentially providing a vector for MeHg entry to the water column and resulting in the exposure of organisms feeding at the sediment surface (Sunderland et al., 2004).

The production of methylmercury depends on the availability of Hg^{2+} to the methylation and on the microbiological activity (Baldi, 1997; Fitzgerald and Mason, 1997; Meili, 1997; Guentzel and Tsukamoto, 2001). The methylation is usually higher at the sediment-water interface, but can also occur in the water column (Wiener and Spry, 1996). Obviously, the amount of methylmercury present is a function of the methylation and demethylation rates (Boudou and Ribeyre, 1997; Meili, 1997) the last process also being mostly microbiological.

As examples of factors affecting the methylation, besides the availability of Hg^{2+}, are also mentioned (Beckvar et al., 1996; Meili, 1997; Post et al., 1996) the oxygen concentration, pH,

redox potential, the presence of sulphate or sulphide, type and concentrations of organic and inorganic complexing agents, salinity and organic carbon. Therefore, by itself, the concentration of inorganic mercury in the environment might not be a good indicator of concentration of existent methylmercury due to the influence of the mentioned environmental variables and of the biological activity.

2.5 Interaction of mercury with organisms: availability, bioaccumulation, biomagnification and elimination

Although no essential role of mercury is recognized in any metabolic pathway, this metal is accumulated in most of the organisms (Meili, 1997). In fact, the concern with bioaccumulation of contaminants in the environment started with mercury, and rose in the middle of the last century with the recognition of some incidents (Barron, 2003).

Methylmercury is readily transferred through the biological membranes and binds strongly to sulphydryl groups of proteins in tissues like muscle. As a result of a depuration rate much slower than for inorganic mercury, the methylmercury has an enhanced accumulation potential (Bloom, 1992; Laporte et al., 1997; Phillips et al., 1997). Then, an increase of the methylmercury fraction along the trophic chain is observed, which in piscivorous fish species contributes to almost all the mercury being found in the form of methylmercury (Joiris et al., 1999; Bowles et al., 2001; Kehrig et al., 2001; Agah et al., 2007).

The accumulation in aquatic organisms by mercury uptake from water alone is defined as bioconcentration. The steps of the uptake process include the transport of mercury to the absorbent epithelium (e.g., the gills), its movement across the diffusion barriers (e.g., mucous, membranes) to the blood and the internal distribution of mercury through the circulatory fluids. The extent of accumulation and the uptake rate are a function of the permeability of the absorbent membrane and of other tissues, which can establish a barrier to the intake and transfer of the contaminant (Barron, 2003). However, the uptake from the diet is generally the predominant route of mercury bioaccumulation in several organisms, being the elimination rate from tissues slow (Tremblay, 1999).

The extent of bioconcentration is quantified by the bioconcentration factor (BCF), defined as the ratio between the mercury concentration in the organism tissue and the concentration in water, in equilibrium conditions. This factor is an estimate of the aptitude to the accumulation of mercury and conceptually can be thought as the volume of water containing the amount of mercury concentrated in 1 gram of tissue of the organism.

Once inside the organism, mercury becomes available to biotransformation, meaning that it might be transformed into other chemical species. The biotransformation can promote the elimination, detoxification, isolation, redistribution or activation of the metal. Although in the case of biological methylation the result is a much more toxic compound, with a higher bioaccumulative ability, sometimes the transformations can also result in less toxic forms. In opposition to the uptake, the elimination results in a decrease of its quantity in the organism. Fish, for example, can eliminate by transport through the gills and secretions. The concentration of mercury in an organism during its lifetime can display a decrease defined as dilution growth (Meili, 1997). This dilution is not considered as an elimination contribution, since the amount of contaminant is not changed due to growth. Despite being used as a synonym, depuration has a different meaning than elimination; the depuration is associated to a peculiar experimental design in which the organism is placed in a non contaminated environment, being the contaminant lost thereby with time.

In phytoplankton, algae and microorganisms, mercury uptake is primarily a passive process that occurs by adsorption to the cell surface either through interaction with functional groups in the cell wall or through adsorptive properties associated with the extra cellular matrices (Beckvar et al., 1996).

The amount of mercury accumulated is a function of the exposure route, availability of mercury and physico-chemical and environmental factors (e.g temperature, pH and concentration of dissolved organic carbon) (Watras et al., 1998). The bioaccumulation includes two distinct processes, the already mentioned bioconcentration, and the biomagnification, defined as the increase in mercury concentration caused by the transfer from a trophic level to a higher level. The biomagnification of mercury occurs even when the concentrations in the system, namely in the water, are low. (Morel et al., 1998; Chen et al., 2000; Kehrig et al., 2002; Yang et al., 2002).

Regarding the relative importance of factors affecting the bioaccumulation of mercury, several doubts remain (Watras et al., 1998). For pH and dissolved organic carbon, Watras et al. (1998) reported positive correlations between these factors and the accumulation of mercury, but others reported negative correlations. These apparent contradictions reflect the complexity and degree of interactions between the multiples effects of the several variables that control the accumulation of mercury.

Several studies point out the importance of the trophic position in the accumulation of mercury. The lower trophic levels can play a major role in the bioaccumulation of mercury, with the high bioconcentration occurring between the water and the phytoplankton. Obviously, factors

that favour the uptake of mercury in the bottom of the trophic chain will contribute to an increase in the efficiency of the transfer to the following trophic levels (Morgan and Mason, 1997).

Generally mercury accumulates in the trophic chain after the transformation of inorganic mercury in methylmercury, mostly in the surface of sediments, where the decay of organic matter also occurs (Lucotte et al., 1999). The particulate organic matter and its bacterial film seem to play an important role in the accumulation of mercury because they constitute a food source to invertebrate biota. Because the phytoplankton is an important primary producer in aquatic systems (and thereby a key element in almost all aquatic trophic chains), the accumulation of mercury in phytoplankton has implications in all aquatic ecosystems. On the other hand, invertebrates like insects and zooplankton, play a key role in the mercury cycle because they constitute the primary diet of several fish species (Verdon and Tremblay, 1999; Kainz et al., 2002; Yang et al., 2002), being a major source of total mercury and methylmercury to higher trophic levels (Tremblay and Lucotte, 1997).

The requirements for biomagnification include an effective uptake of contaminant in the microorganisms at the bottom of the trophic chain, the retention in these organisms and finally the transfer to their predators. Many metals are efficiently accumulated in planktonic bacteria and microalgae, but are not biomagnified: an increase in their concentrations in the biomass is not observed (they often decrease) at the higher levels of the trophic chain. Decisive to the biomagnification behaviour of mercury is the fact that Hg^0, Hg^{2+} and dimethylmercury are not bioaccumulated, in contrast with methylmercury, which is. Dimethylmercury and Hg^0 are not bioaccumulated simply because they are not reactive and thus are not retained in photosynthetic and heterotrophic picoplankton (Morel et al., 1998). Consequently, they diffuse out as readily as they diffuse in. The difference between bioaccumulation of Hg^{2+} and methylmercury is more subtle: $HgCl_2$ and CH_3HgCl diffuse through membranes at about the same rate. Both are also reactive with cellular components and are efficiently retained by microorganisms. However, the efficiency of transfer between a marine diatom and a copepod is four times greater for methylmercury than for Hg^{2+}, which is explained by the fact that Hg^{2+} becomes bound chiefly to particulate cellular material (membranes) of the diatoms which are excreted rather than absorbed by the copepod (Mason et al., 1996). On the contrary, methylmercury is associated with the soluble fraction of the diatom cell and is efficiently assimilated by the copepod. Field data indicate that this difference in the efficiency of transfer between Hg^{2+} and methylmercury also applies to other unicellular microorganisms and their predators (Watras and Bloom, 1992).

In spite of the importance of the diet in the accumulation of mercury in fish (Yang et al., 2002), the contribution of the mercury taken up from the water by respiration cannot be disregarded. Besides factors like the concentrations and mercury species in the water, the temperature and the fish size affect the mercury uptake, by affecting the respiratory rhythm. The relative contributions of the two incorporation vectors (dietary uptake and from water) depend on the season, due to seasonal variations of all the factors previously mentioned, including the diet (Post et al., 1996). In periods of the year that a fish feeds on organisms with lower mercury levels, which can conjugate with low temperatures, the route of uptake through the water will gain importance. Post et al. (1996) alert to the fact that the studies of the relative importance of the two exposure routes are based, most of the times, in piscivorous fish of an appreciable size (which present lower respiratory rhythms and feed on preys already with high mercury levels), disregarding a lot of fish species and also young piscivorous fish, which are small, with higher respiratory rhythms and that feed on invertebrates or algae with substantially lower mercury levels. In brief, in piscivorous fish, the major exposure route seems to be the diet through contaminated fish, while in fish that feed on plants, a preponderance of the incorporation from water appears to be observed (Post et al., 1996). Considering this, the trophic structure arises as a source of variability of the rates of mercury consumption and of the mercury concentrations between fish (Wong et al., 1997).

The incorporation of mercury in organisms depends on the availability of the metal, i.e. on its ability to bond and/or cross biological barriers that separate the organisms from the environment where they live. The physico-chemical conditions of the ecosystems can induce structural and functional modifications in organisms, which can affect the availability of metal and hence its bioaccumulation and toxicity (Laporte et al., 1997). The aquatic chemistry of the system (pH, salinity, concentration of ligands) influences the uptake of inorganic mercury and methylmercury by phytoplankton (which takes place by Hg passive diffusion of neutral species through membranes) since controls the mercury species present (Morgan and Mason, 1997). For example, if phytoplankton is exposed to complexes CH_3Hg-ligand, no important role is recognized for the complexing ligand, since the complex crosses the lypophilic membrane and dissociates due to the change of chemical environment. Mercury then binds to other ligands or reactive sites within the cell (Morgan and Mason, 1997). For this reason, when organisms of the upper trophic level ingest phytoplankton, the ligands do not show a preponderant influence on the assimilation. Therefore, the aquatic chemistry affects only the uptake of the bottom of the food chain, with no influence on the subsequent transfer to consumers.

The availability to organisms of mercury associated to sediments depends not only on the species present but also on the sediment chemistry (salinity, redox and pH), transformation phenomena (e.g. methylation), association to dissolved organic matter (Barron, 2003) and presence in sediment of sites to bond the metal. An increase in the concentration of iron oxides or organic materials reduces the availability of mercury to organisms. Low pH values increase the mobilization of methylmercury from the sediments, enhancing the exposure of aquatic organisms (Stemberger and Chen, 1998). Benoit et al. (1998) reported low methylmercury fractions in the sediment (between 0.1 and 0.5% of the total mercury) and positive correlations between methylmercury, organic matter content and total mercury, negatively correlated with sulphide content.

2.6 Mercury levels in aquatic systems

In order to evaluate the anthropogenic contribution of contaminants in the environment, it is useful to estimate the concentration that would be expected in the absence of human induced contamination, the background concentrations (BC). Although, it is nowadays recognized that BC are highly specific of the concerned locations and therefore the comparison with levels from other areas is unadvisable because the influence of natural sources of Hg to a specific location depends on the peculiarities of its location. Regional background/reference concentration should then be derived from expert consensus, historical data or through statistical treatment of solid surveys. Besides being site specific, the BC in biota are also particular of each species. The task of deriving BC is even harder for trace elements like mercury, which has a unique and very complex biogeochemical behaviour (with exchanges with atmosphere). A classical example regarding the establishment and interpretation of background levels is the dissolved Hg, which first depends on the suspended particulate matter content (Lanne, 1992).

Table 2.1 shows the levels of total mercury in some studies, as well as the percentage of methylmercury. The concentrations vary considerably and can be attributed to hydrogeochemical characteristics and/or contamination of the various systems. For some studies is also recognisable the increase of Hg levels within the trophic chain.

The fraction of methylmercury in sediments is in general very low, being normally below 1% (Benoit et al., 1998; Farago, 2000). Ramalhosa (2002) concluded that concentrations of methylmercury represent 0,008-0,096% of total mercury, in sediments until 50 cm depth, in a very contaminated area of the Ria de Aveiro.

Table 2.1 - Levels of total Hg (μg g^{-1} unless otherwise mentioned) and methylmercury (%) in various aquatic systems.

Local	Matrix	Hg_{total}	MeHg (%)	Reference
Amazon River (Brazil)	Phytoplankton	0,066	15	Roulet et al., 2000
	Zooplankton	0,162 – 0,194	14 - 71	
Mediterranean	Sardines	0,19 – 0,75	85 - 97	Joiris et al., 1999
Guanabara Bay-Brazil	Water (dissolved)	0,7 – 5,2 ng L^{-1}	-	Kehrig et al., 2002
	Water (SPM)	0,0607 – 0,3800	-	
	Bivalvia	0,0382	33	
	Planktivorous fish	0,0154	54	
	Piscivorous fish	0,1995	98	
Lake in Scotland	Water (not filtered)	14.7 ng L^{-1}	--	Yang et al., 2002
	Zooplankton	0,2344	--	
Coast of Galicia	Seawater	< 1.0 – 73.5 ng L^{-1}	--	Beiras et al., 2002
	Sediment	0.050 – 2.73 μg g^{-1}	--	
	Mussels	0.101 – 1.11 μg g^{-1} dw	--	
Marmara Sea, Turkey	common sole (Solea solea)	0.329 ± 0.0224 ww mean ± SD	--	Keskin et al., 2007
Venice Lagoon	Sediment	0.1 - 3.4 μg g^{-1}	--	Berto et al., 2006
	Gastropods	0.3 - 1.3 μg g^{-1} dw	--	
Spanish North-Atlantic coast	Mussels	0.09 – 0.29 μg g^{-1} dw	--	Besada et al., 2002
Persian Gulf	Fish species	0.050 - 0.542 μg g^{-1} dw	64 - 100	Agah et al., 2007
Ria de Aveiro	Water (dissolved)	1.0 – 275 ng L^{-1}	--	Coelho et al., 2005
	Water (SPM)	0.3 – 25.8 μg g^{-1}	--	
	Sediment	0.1 – 51.7 μg g^{-1}	--	
	Macroalgae	0.02 to 2.1 μg g^{-1} dw	< 20% *	
	Bivalve (Scrobicularia plana)	0.019 – 1.8 μg g^{-1} ww	5 - 26% *	Coelho et al., 2006
	Sea bass	0.03 – 1.7 μg g^{-1} ww	--	Abreu et al., 2000

dw: dry weigth; ww: wet weight; * determined as total organic mercury

2.7 Toxicity

The toxicity of mercury to aquatic organisms is affected by both biotic and abiotic factors, including the speciation (inorganic vs. organic), the environmental conditions (e.g., temperature, salinity and pH), the sensitivity of different species of organisms to the metal, the life history stage and the tolerance of individual organisms (Beckvar et al., 1996). Considering the speciation, the organic forms of mercury are more toxic than the inorganic ones. As an example, the toxicity (defined by LC_{50} values) of methylmercury chloride to the red algae *Plumaria elegans* is 70 times higher than the toxicity of mercury(II) chloride (Clark, 2001).

The toxicological effects of mercury include neurological damages, reduction of reproduction capacity, growth inhibition, development abnormalities and changes of responses in terms of behaviour (Wiener et al., 2003). Concerning the exposure of adult fish to methylmercury, the neurotoxicity seems to be the main effect, taking into account the observed coordination faults, feeding incapacity, lack of appetite, diminished response capacity, lethargy, abnormal movements and brain lesions (Wiener and Spry, 1996).

Fish always lived in environments containing mercury. Despite the fact that fish have mechanisms of protection against inorganic mercury, they seem to have few defences against methylmercury. The association of methylmercury to proteins of the skeletal muscle has the advantage of reducing the exposure of the brain, which is the organ more sensible to the adverse effects of methylmercury (Wiener and Spry, 1996).

2.7.1 Health effects on humans

The mercury threat is demonstrated by its third position occupied in 2005 in a priority list of hazardous substances (for the potential danger to human health due to its toxicity) presented by ATSDR (Agency for Toxic Substances and Disease Registry). The extent of the health effects in an exposure to mercury depends on the exposure period, mercury form of concern and the exposure route.

The primary route of human exposure to methylmercury is the diet, more concretely the consumption of contaminated fish (USEPA, 2001; Zahir et al., 2005). According to studies cited by Drash (1994), besides the diet, the dental amalgams also represent some risk; the air and water contribute to a possible exposure to humans only in cases of local contaminations. Thereby, communities consuming large amounts of contaminated fishery products are subject to neurological damages and damages in fetus. The best-known incident of the evidence of the human health risks in mercury poisoning, occurred in Minamata, precisely due to the consumption of methylmercury biomagnified along the trophic chain in a fishermen community (WHO, 1990; Hamada and Osame, 1996). In the 1950's some residents started to display complaints related to the peripheral and central nervous system. In 1956, a research group reported an initial suspicion of a metal poisoning, at the time presumably through the ingestion of contaminated fish and shell-fish, but only 16 months later it was suggested that Minamata disease symptoms resembled those of organic mercury poisoning. The contamination was caused by the release of methylmercury in the industrial wastewater from an industry producing acetaldehyde. The chemical reaction used mercury sulphate as a catalyst and a side reaction of

the catalytic cycle led to the production of small amounts of methylmercury. Mercury was released to Minamata Bay through the industrial wastewaters, resulting in an accumulation of 70-150 ton of mercury in a relatively small bay (382×10^4 m^2) (Kudo et al., 2000).

There is no doubt that the reactivity of mercury with amino, thiol or sulphydryl groups of proteins is a major mechanism for the biological activity of mercury (Schäfer et al., 1999). Nevertheless, since proteins with sulphydryl groups occur in both intracellular and extracellular membranes and organelles, and because most sulphydryl groups play an integral part in the structure or function of most proteins, the precise target(s) for mercury is not easily determined, if indeed there is a specific target (ATSDR, 1999).

The central nervous system is the main target of methylmercury in human adults (Crespo-Lopez et al., 2007). The sensorial functions, vision and audition, together with certain zones of the brain, specially the cerebellum, related to coordination, are the more affected functions (WHO, 1990; Zahir et al., 2005). Besides the neurological damages, mercury has also been found to be a causative agent of various other sorts of disorders, including immunological, cardiac, motor, reproductive and even genetic (Zahir et al., 2005; Crespo-Lopez et al., 2007). The pre-natal exposition to methyl mercury also causes severe damages in the central nervous system, namely delays in the psychomotor development. The inhalation of a certain level of organic mercury or metal mercury, and the exposition to organic mercury by any route can be fatal for human beings and animals (ATSDR, 1999).

2.8 Mercury regulations

The toxicity characteristics of mercury, together with its high persistence and bioaccumulation made it important to create legislation in order to establish the limit values and the quality aims for mercury discharges. Therefore, with the present legislation, it is possible to control the emissions of this metal into the environment, in particular to the aquatic system.

In what concerns the chlor-alkali electrolysis sector, the “Decreto-Lei n.º 431/99 de 22 de Outubro” establishes a maximum value of 50 $\mu g\ L^{-1}$ of Hg for the effluents of this kind of industry. It also establishes the quality criteria, as for example the maximum levels of mercury in the waters affected by the discharges and in the fish chosen as indicators. Besides that, for other sectors (“Decreto-Lei n.º 52/99 de 20 de Fevereiro”) the law also takes into account the amount of Hg discharged per kilogram of Hg used, depending on the type of industry. For the vinyl chloride industry, besides the previous limits, the production capacity is also considered (0.1 $g\ t^{-1}$).

The “Decreto-Lei n. 236/98 de 1 de Agosto” defines the demands for a range of different water uses. Regarding the superficial freshwaters for the production of drinking water, the maximum recommended level is 0.5 μg L^{-1}, with a maximum limit of 1.0 μg L^{-1}. There are no limits to the mercury concentrations in what regards superficial, brackish or coastal waters used as fishery resource.

Due to the mercury accumulation in organisms, it was necessary to establish the limit of Hg in drinking water and in foodstuff likely to be contaminated as fishing products. The “Decreto-Lei n. 306/2007 de 27 de Agosto” establishes a maximum acceptable limit for drinking water of 1 μg L^{-1}. The “Regulamento n.º 466/2001” limits the maximum level of 0.5 mg kg^{-1} in fish products (fresh weight). However, for some species (sea bass, eel, tuna fish, among others) this limit is of 1.0 mg kg^{-1}. The “Regulamento (CE) N.º 221/2002” changes the previous one and adds the species *Coryphaenoides rupestris* and *Hoplostethus atlanticus* (imperator).

3. Methods

This chapter describes the experimental methods used in the current study, including sampling, laboratory operating procedures of sample treatment and analysis and the adopted procedures of quality assurance and quality control (QA/QC). Detailed information on the sampling design will be described in the relevant chapters.

3.1 Cleaning procedures

Efforts were made to use ultra-clean protocols during the entire process of sample collection and handling, in order to minimize the possibility of sample contamination (procedures adapted from Bloom, 1995; USEPA, 1996; Stoichev et al., 2006a). The cleaning of material is very important because it can compromise irreversibly all the work. All the material contacting with samples (from sampling to analysis, including storage and pre-treatment) and reagent solutions was previously decontaminated. The procedure for material cleaning starts by washing with tap water followed by immersion for at least 24h in a bath containing 5% Decon®. Next, it was washed with distilled water and immersed for at least 24h in 4 mol L^{-1} HNO_3 and finally thoroughly rinsed with distilled water. Material for water samples handling and preservation was cleaned with concentrated HNO_3 before soaking in a 4 mol L^{-1} HNO_3 bath, used exclusively for water samples material. After the immersing in 4 mol L^{-1} HNO_3, this material for water samples was thoroughly rinsed with ultra-pure water (>18 MΩcm) obtained from a Millipore Milli-Q model 185 system.

Material for dissolved organic carbon and particulate organic carbon analysis was additionally washed with sulphochromic acid mixture and rinsed with abundant ultra-pure water.

3.2 Sampling

Sampling is an integral part of the measurement process that is often neglected in terms of the errors that can generate. Mistakes during the sampling process inevitably lead to erroneous results, which cannot be corrected afterwards. Considering the remarkable progresses in analytical methods, including sophisticated instrumental features, sampling is increasingly becoming the quality-determining step. Most of all, all efforts must be addressed to guarantee representativity of samples and procedures to avoid contamination or losses must be adopted.

3.2.1 Water column

The characterization of the water column was performed by sampling at two depths. In the channel cross-section, samples were collected with a Van Dorn® teflon bottle, while in the ocean Niskin bottles were used. During sampling, and when appropriate, the "clean hands/dirty hands" procedure was followed to discriminate the contamination risk. Before the collection, the Van Dorn bottle was rinsed with a first collection of “sample”, which was discarded. The samples were transferred to acid-cleaned PET bottles (Fadini and Jardim, 2000; Stoichev et al., 2006a) and after rinsing two times with the sample prior to filling, were bagged and transported in coolers to the laboratory. Temperature, salinity and pH were also measured directly on board with a conductivity/salinity meter YSI model 33 and pH with a WTW 538 pH meter, respectively.

To the collection of suspended seston material, 63 µm and 200 µm plankton nets were used working in conjunction with attached flow meters. Trawl surveys during some minutes were carried out at two depths and at some distance of the boat. Frequently, material collected in the nets contained macroalgae, plants or other debris that were always removed. The remaining part, which consisted mainly of plankton and suspended sediments was stored in polypropylene containers and refrigerated until transport to the laboratory.

3.2.2 Sediment

Collection of sediment was carried out using a quantitative grab sampler Smith-McIntyre with a unitary cell of 0.1 m^2. Samples were stored in polyethylene bags and refrigerated until transport to the laboratory.

3.2.3 Biota

Ichthyofauna was sampled in the ocean in two sampling stations by beam trawl at depths approximately between 10 and 22 meters. Bivalves were sampled by trawl with a bivalve dredge. Specimens were stored in polyethylene bags and refrigerated until transport to the laboratory.

3.3 Sample treatment and analysis

3.3.1 Water column

Samples were processed within a few hours after sampling. The specificities of treatments for characterization of dissolved and particulate constituents of water column are described in the respective section of each determination.

Suspended particulate matter

Water samples (500-1000 mL) were filtered through pre-weighed, 0.45 µm pore size Millipore filters. Filters were re-weighted after heating, overnight, at 60 °C and stored at 4 °C until mercury analysis. The concentration of suspended particulate matter (SPM) was determined by the ratio between the mass of dry material retained in the filter and the volume of water filtered.

Mercury in water samples

Dissolved mercury

For dissolved mercury analysis, the filtrates obtained in the separation of dissolved and particulate phases (see previous paragraph) were collected to acid-cleaned borosilicate glass bottles (USEPA, 1996), acidified with mercury-free HNO_3 to pH < 2 and stored at 4 °C prior to analysis. Reactive mercury was analysed by cold vapour atomic fluorescence spectrometry, CV-AFS (PSA cold vapour generator, model 10.003, associated with a Merlin PSA detector, model 10.023), using $SnCl_2$ as a reducing agent (2% in 10% HCl). This mercury fraction includes mostly ionic (Hg^{2+}) plus dissolved gaseous mercury, but also the mercury that is weakly bound to inorganic and organic dissolved complexes.

For total dissolved mercury quantification, filtered water sub-samples (50 mL) were digested by addition of 500 µL of a saturated solution of potassium persulphate, assisted by 30 minutes irradiation with a 1000 W UV lamp; following irradiation, the excess of oxidant was reduced with 37.5 µL of hydroxylamine solution 12% (w/v) (Mucci et al., 1995). After this photochemical oxidation, total dissolved mercury was determined using the same instrument used for reactive mercury quantification. Procedure and reagents contamination was followed by analysis of filtrate blanks and ultra-pure water. In both the reactive and total dissolved mercury determinations, the equipment was calibrated with at least five acidified standards (from 2 ng L^{-1} to 30 ng L^{-1}) prepared by dilution of a 1000 mg L^{-1} "BDH" mercuric nitrate standard solution.

Mercury in SPM

The particulate total mercury was analysed by CV-AFS after HNO_3 digestion. Acid digestion of filters with SPM (see *Suspended particulate matter* in previous section) was carried out by adding 50 mL of nitric acid (4 mol L^{-1}, p.a.) in a borosilicate glass beaker (covered with a watch glass), standing overnight at room temperature before being heated in a sand bath for 4 hours at 60 °C (Monterroso et al., 2003). The CV-AFS was calibrated with at least five acidified standards (from 0.05 µg L^{-1} to 0.5 µg L^{-1}) prepared by dilution of the 1000 mg L^{-1} mercuric nitrate standard solution.

Organic carbon in water samples

Dissolved Organic Carbon

For dissolved organic carbon (DOC) determinations, 1-2 L aliquots of sample were filtered, with a pre-combusted (6 hours at 450 °C) glass fibre filter (GF/C). All the glassware was cleaned with sulphochromic acid mixture and blank analysis were performed. DOC determinations were made by analysis of the filtrate by an automated procedure (Alliance, model Evolution II) in which the sample is acidified and the inorganic carbon, as CO_2, removed by N_2. Subsequently, a buffered persulphate solution was added and the solution was irradiated in an UV digester (Skalar, model SA 5550). A hydroxylamine solution was then added and the carbon dioxide generated diffused through a gas silicone membrane. A weakly buffered phenolphthalein indicator solution was used as a recipient stream. The colour intensity of this solution decreased proportionately to the change in pH caused by the absorbed carbon dioxide gas (Lopes et al., 2006a). The instrument was calibrated using a series of standards from 1.0 to 5.0 mg L^{-1} of carbon prepared with potassium hydrogen phthalate in ultra-pure water.

Particulate Organic Carbon

The particulate organic carbon (POC) was determined in the particles retained in the filters used for DOC determinations. After collection of SPM in the filters, they were stored at -18 °C until analysis. The estimation of POC was performed by potassium dichromate chemical oxidation in presence of sulphuric acid, followed by titration of the excess of oxidant with an iron solution, as suggested by Kramer et al. (1994). The filters were placed in Erlenmeyer flasks, 2 mL of orthophosphoric acid was added and the Erlenmeyer flasks (covered with watch glasses) were heated in a sand bath for 30 minutes at 100 – 110 °C. Next, 10 mL of a oxidant mixture (4.84 g of potassium dichromate and 500 mL of sulphuric acid per 1 litre of solution) was added to each Erlenmeyer flask and heated again (covered again with the watch glass) in the sand bath for 30 – 60 minutes. After cooling to room temperature, they were titrated with a 0.1 mol L^{-1} Fe(II) solution after addition of 50 mL of distilled water and 2 drops of ferroin indicator. Blank filters were also processed as samples. The Fe(II) solution was standardized by titration of 5 mL a glucose solution corresponding to 1500 µg of C. The glucose solution was prepared daily and titrated after digestion by the some procedure used for the filters. Ten millilitres of the oxidant mixture was also titrated with the Fe (II) solution. Details of the quantification of POC based on the titrimetric results can be found in Ramalhosa (2002).

Chlor a

Chlorophyll a (Chl a) concentration was estimated spectrophotometrically through a trichromatic method (Parsons et al., 1984; Arar, 1997). Aliquots of sample (1 L) were filtered through a 0.45 µm Millipore cellulose acetate membrane filter. After filtration, pigments on filters were extracted during 12 hours, in darkness at 4 °C, in polyethylene tubes with 10 mL of acetone (90%). In the next day, the absorbance of the supernatant at 750, 664, 647 e 630 nm were measured in a UV-Vis spectrophotometer (Shimadzu, UV-2101 PC) with a 1 cm cell. The measurement at 750 nm is used to correct for turbidity, being subtracted from the absorbance at each of the other three wavelengths, to obtain the concentration of chlorophyll a in the extracts. Details of the quantification of chlorophyll a based on the trichromatic absorbances of the extracts can be found in Arar (1997) and Monterroso (2005).

3.3.2 Sediments

Total mercury concentrations

Sediment samples were freeze-dried (Christ Alpha 1-4 lyophilizator), homogenized and manually sieved using a 1 mm size nylon sieve. Samples were directly weighed (300 – 500 ± 0.1 mg) into pre-cleaned combustion boats and total mercury levels quantified using atomic absorption spectroscopy (AAS) following thermal decomposition of the sample using an Advanced Mercury Analyser (AMA, LECO 254). The sample is firstly dried at 120 °C prior the combustion at 680–700 °C in an oxygen atmosphere. The mercury vapour is collected in a gold amalgamator and after a pre-defined time (120–150 sec) the gold amalgamator is heated at 900 °C. The released mercury is transported to a heated cuvette (120 °C) and then analysed by atomic absorption spectrometry (AAS) using a silicon UV diode detector (more details on the methodology can be found in Costley et al. (2000), Cizdziel et al. (2002) and Haynes et al. (2006)). Operational conditions used included a drying time: 10 sec; decomposition time: 150 seconds; waiting time: 40 seconds. The instrument was calibrated with 8 standards (from 0.5 ng to 40 ng) obtained by analysis of standards solutions prepared by dilution of a 1000 mg L^{-1} mercuric nitrate standard solution.

Fine fraction content

The percentage of fine particles in sediments was determined gravimetrically by wet sieving about 5.0 g of dried sediment through a 63 µm sieve.

Carbon content

Total carbon content of the sediments was determined in four replicate samples with a LECO model 932 CHNS analyser that measures carbon by means of an infrared detection system.

3.3.3 Material collected with plankton nets

Total mercury concentration

Seston samples for mercury analysis were frozen and freeze-dried after collection. Homogenized solid samples were directly weighed (50 – 500 ± 0.1 mg) into pre-cleaned combustion boats, and total mercury concentrations quantified by atomic absorption spectroscopy following thermal decomposition of the sample (using an Advanced Mercury Analyser, LECO AMA-254). Calibration of the equipment was detailed in the description of mercury determinations in sediments.

Organic mercury concentrations

Organic mercury in seston (quantified as total organic mercury) was determined via an extraction process involving acid leaching with a mixture of KBr, H_2SO4 and $CuSO_4$ and extraction of organic mercury to toluene (Válega et al., 2006). About 50–200 (± 0.1) mg of sample were weighed into 50mL polypropylene centrifuge tubes and then 5mL of KBr (18%) in H_2SO_4 (5%) and 1mL of $CuSO_4$ (1 mol L^{-1}) were added to the tube. The tubes were held at room temperature for 15 min and then treated with 5 mL of toluene, followed by vigorous agitation for 15 min, to extract organic mercury. The organic phase was separated by centrifugation (4000 rpm for 15 minutes) and then 3 mL of the organic extract was decanted to glass vessels and stored. The extraction process was repeated twice more and each time the organic extract was retained. The organic mercury compounds retained in the toluene were back-extracted into an aqueous sodium thiosulphate solution 0.002 mol L^{-1} (5 mL). Quantification of liquid aliquots (500 µL ± 3 µL) was carried out using an Advanced Mercury Analyser (AMA) LECO 254, in the aqueous medium after back-extraction with thiosulphate. Calibration of the equipment was detailed in the description of mercury determinations in sediments. Procedural blanks were carried out for quality assurance purposes.

Carbon and nitrogen content

As for sediment samples, total carbon and nitrogen contents in seston was determined in four replicate samples with elemental analyzer (Leco, model 932).

3.3.4 Biota

The fish and bivalves captured were transported to the laboratory and muscle and liver in fish were separated, freeze-dried and homogenized. For bivalves, the entire body was used.

Total mercury concentrations

For the determination of total mercury in biota, homogenized tissues samples were directly weighed (50 - 300 ± 0.1 mg) into pre-cleaned combustion boats, and total mercury concentrations quantified by atomic absorption spectroscopy following thermal decomposition of the sample (using an Advanced Mercury Analyser LECO AMA-254). Calibration of the equipment was detailed in the description of mercury determinations in sediments.

3.4 Quality Control and Quality Assurance

Analytical methods developed for environmental analysis include a number of single operations, each of them must be considered as a potential source of errors. The total uncertainty budget associated to the final results can be considered as a sum of different contributions from different steps in which the analytical method can be divided. The largest contributions arise usually for at least four critical steps, including sampling, storage, treatment and instrumental analysis.

$$u_T = (u^2_{sampling} + u^2_{storage} + u^2_{treatment} + u^2_{analysis} + \ldots + u^2_{data\ treatment})^{½}$$

In the present study, in every one of the analytical procedures used, all efforts were aimed to minimize all the contributions to the uncertainty of results. Depending on the specificity of the methods, different sources of uncertainty (not necessarily independent) can contribute to the different components of final uncertainty and should be identified and considered:

- Sampling and sub-sampling;
- Storage conditions;
- Instrument effects;
- Reagent purity;
- Departures from assumed reaction stoichiometry (departures from expected stoichiometry in particular reactions of a analytical process);
- Measurements environmental conditions (laboratory temperature, humidity, etc);

- Sample matrix effects and interferences (related to recovery of an analyte from complex matrix or an instrument response that may be affected by composition of the matrix);
- Computational effects (truncation, rounding and inappropriate selection of calibration models);
- Blank correction (especially in trace analysis, where the uncertainty of the blank and appropriateness of the blank correction needs to be evaluated);
- Operator effects;
- Random effects.

3.4.1 Analytical figures of merit

Monitoring of analytical figures of merit such as accuracy, precision, limits of detection (LOD) and linearity of the working range is an indispensable tool to judge the performance of the methods and therefore the reliability of the results.

The accuracy can be evaluated by the analysis of Certified Reference Materials (CRMs), when available, which should have a matrix as similar as possible to the samples. Considering that it is unreal to considerer a perfect similarity between the matrixes, the analysis of fortified samples also arises as a valuable tool in the analysis of recoveries. However, it should never be considered as a substitute to the use of CRMs, because in the case of fortification of samples, the equilibrium between the analyte and the added substance is not known. In fact, it must be noted that most methods of fortification add the analyte in such a way that it will not be as closely bound to the sample matrix as it would be if it was present naturally. Consequently, recovery determinations obtained by fortification of samples can be expected to be over-optimistic, and should be used in a conservative way: if quantitative recoveries in spiking experiments are obtained, this does not mean that the same efficiency will be achieved on unknown samples, but if quantitative recoveries are not obtained, it is sure that no quantitative extraction will be achieved on unknown samples. Overall, spiking is a very useful and efficient procedure to track losses during analyses and to check the efficiency and accuracy of some steps of the analytical methods.

Concerning the precision, this figure of merit can be estimated by the dispersion of replicate results. The linearity of the working range can be assessed by the use of regression models and establishing quality objectives for the correlation coefficient, like previous mentioned.

Finally, it is very important to determine the lowest quantity of a substance that can be statistically distinguished from the noise signal, from the baseline or from the absence of that substance (blank value) in certain experimental conditions. The practical method to define the limit of detection (LOD) can be to consider the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank. When the blank signals are undistinguishable of the baseline, the determination of the LOD can be performed based on parameters of the calibration curves. The instrumental detection limit is an useful tool to evaluate the instrument performance but can grossly underestimate the ability to quantify low concentrations. In fact, the manipulation of sample during an analytical method can be quite more than just doing a reaction or submitting it to direct analysis. In these cases, the method detection limit (MDL) can be very useful to define the lowest quantity of a substance that can be distinguished from the absence of that substance. This detection limit is based on samples which have gone through the entire sample preparation scheme prior to analysis and can be determined by replicate analysis of blanks or low-level samples. Most of all, it should be clearly stated the approach used to estimate the LOD.

In this study, the adopted procedures of quality assurance and quality control (QA/QC) included analysis of CRMs, replicates, calibration curves, calibration checks, spiking experiments. In all analysis, processing of blanks as samples (procedural blanks) was also performed to assess and control contamination. Acceptance criteria requirements for methods figures of merit were established to meet pursued quality objectives.

3.4.2 Acceptance criteria

In the present work, quality objectives were defined to establish the accepted precision, accuracy of results and quality of calibrations. The following acceptance criteria were adopted, or else the analysis were repeated, whenever possible:

- Re-analysis of imprecise replicate results (relative standard deviation above 10%).
- Strict demands in the quality of calibration were also imposed in all modelling and data treatment of instrumental responses. Recalibrations were performed for correlation coefficient lower than 0.995 and for instrument response drifts higher than 10%.
- Results of sample analysis determined together with CRMs results significantly different (p=0.05) from certified values were not accepted.

- High or unstable blank results were always investigated and determinations were not considered when quality of the results was questionable.

3.4.3 Quality control of laboratory operating procedures

Dissolved mercury

The CV-AFS instrument was calibrated daily with at least five acidified standards and a standard was analyzed between three samples to check for instrument drift (ratio number of samples: number of standards analysed never lower than three).

In the determination of dissolved mercury, in order to examine for any possible contamination during the filtration procedure, blank solutions (100 mL of ultra-pure water) were acidified with 50 µL concentrated HNO_3 (Merck, mercury-free) and were filtered in between the water samples through the same filtration unit used for those samples.

Blank levels in the filtrate blanks were always below the detection limit. For determinations in the tidal cycles samples, the precision of duplicates expressed as the relative standard deviation was always lower than 20% (lower than 10% in 78% of the determinations). For determinations in coastal waters, mean relative standard variation for dissolved mercury determinations were 14 ± 2 % (n= 78) and 6 ± 1 % (n= 89) for reactive and total mercury respectively (95 % confidence).

A spiking study was performed to evaluate the recoveries of the determination of total dissolved mercury. As stated before, it is important to keep in mind that spiking may fail to give a true indication of native analyte recovery in the samples, being the conditions under which spiking experiments should be carried out still strongly debated within the scientific community. A water sample (salinity= 35.9) was filtered, acidified, analysed and mercury was added at two concentration levels (20 ng L^{-1} and duplicate of 10 ng L^{-1} spike). Recoveries ranged between 92 and 110%, supporting the performance of the method.

In parallel with the study obtained by fortification of samples, a stability experiment was also carried out to investigate the holding time of the acidified samples at 4°C in glass bottles. The concentrations of total dissolved mercury in the control and in the fortified solutions during 14 days of storage are presented in Figure 3.1, with no important losses observed during this period. In addition, agreement between average concentrations on replicate spikes (among 10 ng L^{-1} spikes, R1 and R2) was always lower than 6%.

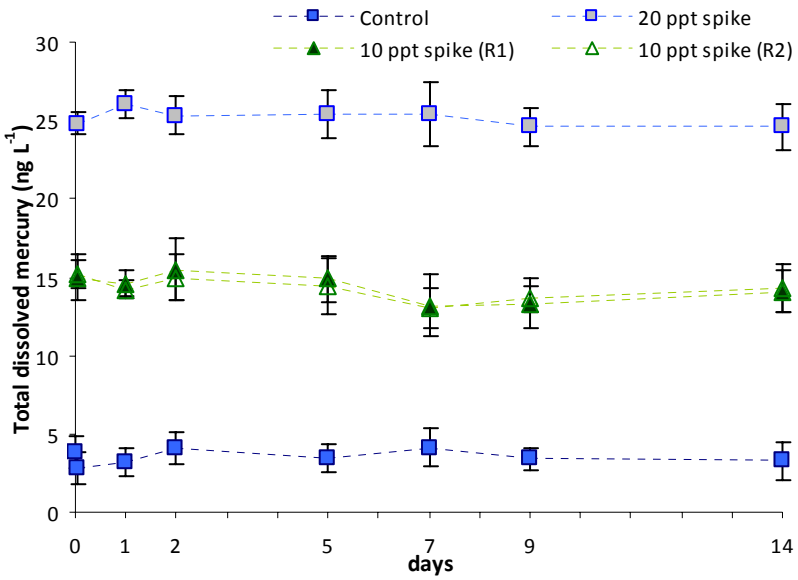


Figure 3.1 - Total dissolved mercury concentrations in control and fortified samples during 14 days.

Mercury in SPM

Calibration and calibrations checks for analyses of SPM digestions by CV-AFS were similar as for dissolved mercury analyses. Procedural filter blanks were processed as samples. Mercury levels in these blank filters ranged between 3.5 and 9.4% (average of 7.3%) of the typical content in samples filters (0.10 - 0.20 µg of mercury). The precision for particulate mercury determinations ranged between 0.4 and 13%.

Quantifications with Advanced Mercury Analyser

The equipment response was checked daily (in the beginning of the analysis and at the end) with analysis of CRMs of similar matrix to the samples. Correction of results was performed according to recoveries obtained for CRMs. This procedure intends to correct daily variation of equipment response and decay of accuracy due to poisoning of instrument catalyst. To control memory effect between samples, blank analyses were always performed between samples. No differences were found between precision for different matrixes ($p<0.05$). Accuracy of quantifications was evaluated by analysis of CRMs being the results presented in a subsequent section.

Besides the analysis of CRMs, the participation in intercomparison exercises allowed to assess the performance and accuracy of analytical procedures. Furthermore, the results of these exercises can be used to identify analytical difficulties in the laboratories and initiate remedial actions, being also useful to characterize trends of analytical performance in the determination of

elements over the years (ISO, 1997). The Z-scores obtained in intercomparison exercises organised by the Marine Environmental Studies Laboratory of the International Atomic Energy Agency were 0.2 for total mercury in marine sediment (IAEA-158 TM), 1.13 for total mercury in tuna fish homogenate (IAEA-436) and 1.51 in the quantification of organic mercury in the same tuna fish homogenate. The achievement of Z-scores between -2 and 2 indicate a satisfactory performance in the quantification of mercury in these matrixes.

DOC

The instrument was calibrated daily with at least five standards and a standard was analyzed every five samples to check for instrument drift. Procedural blanks were always undistinguishable from the baseline. Method performance was evaluated by analyses of samples spiked with ascorbic acid and caffeine and recoveries ranged between 103 and 113% (Lopes et al., 2006a). Detection limit (estimated by parameters of the calibration curves) ranged between 0.23 mg L^{-1} and 0.37 mg L^{-1}.

Carbon and nitrogen contents and Fine fraction content

The median relative standard deviation for the determinations of the percentage of fine particles was 8% (n=50) and was always less than 18%. The relative standard deviations for total carbon (n=140) and total nitrogen (n=96) determinations had a median of 6% (always less than 12%). Reference materials Eurovector E11036 and Eurovector E11037 were analysed and recoveries were found to be 94 ± 9 % (standard deviation) and 105 ± 8 % (standard deviation) for total carbon and total nitrogen, respectively.

Certified Reference Materials

CRMs are a very helpful tool to evaluate a large part of the measurement process. Difficulties were found in obtain a complete validation data set for all analytes due to the lack of suitable CRMs, but they were used whenever possible. The CRMs used were obtained from the National Research Council of Canada (estuarine sediment, MESS-2 and MESS-3; harbour sediment, PACS-2; lobster hepatopancreas, TORT-2), from Community Bureau of Reference (plankton, BCR-414) and from International Atomic Energy Agency (Fucus sp. Homogenate, IAEA-140TM). In Table 3.1, the results obtained in the analysis of CRMs are presented, as well as the certified values and the associated uncertainties.

Table 3.1 - QA/QC assessment based on the analysis of certified reference materials.

	Certified concentration ($\mu g\ g^{-1}$)	**Obtained concentrations ($\mu g\ g^{-1}$)**		
	(95% CI)	*mean*	*95% CI*	*n*
PACS-2	3.04 (3.24 – 2.84)	2.96 *	3.06 – 2.86	4
Harbour Sediment		2.99 * *	3.05 – 2.93	12
MESS-2	0.092 (0.083 - 0.101)	0.0899 * *	0.0855 - 0.0943	10
Marine sediment				
MESS-3	0.091 (0.082 - 0.100)	0.0934 *	0.0830 - 0.104	4
Marine sediment		0.0898 * *	0.0890 – 0.0906	24
TORT-2	Total Hg: 0.27 (0.21 - 0.33)	0.269 * *	0.267 - 0.271	89
Lobster hepatopancreas	Org Hg: 0.152 (0.139 - 0.165)	0.154	0.150 - 0.158	6
BCR-414	0.276 (0.258 - 0.294)	0.202 * *	0.200 - 0.204	28
Plankton				
IAEA-140TM	Org Hg: 6.26×10^{-4}	6.21×10^{-4}	$5.44 \times 10^{-4} – 6.98 \times 10^{-4}$	7
Fucus sp. Homogenate	($5.19 \times 10^{-4} – 7.33 \times 10^{-4}$)			

(mean; 95% confidence intervals, CI; number of replicates, n)

* AFS following 4M HNO_3 digestion ; * * AAS following thermal decomposition of the sample

The obtained results were compared with the certified values following the indications of an application note from European Reference Materials® (Linsinger, 2005). Briefly, the difference between the measured results and the certified values are compared with the expanded combined uncertainty (obtained by combination of the individual uncertainties of the certified and measured results). No significant differences were found for all CRMs, except for BCR-414. The agreement between the certified and the measured concentrations can also be visualized by the distribution of Z-scores for the main CRMs, presented in Figure 3.2, corroborating that laboratory performance in the analysis of these matrixes can be considered satisfactory.

Control charts are useful tools for recording internal quality control data and also to prove quality and proficiency of results. As an example, in Figure 3.3 the control chart of total mercury determinations in the CRM TORT-2 is presented, where C_{CRM} is the certified concentration and $u\Delta$ is the combined uncertainty of result and certified value. No important trends are observed, being all the results within the control limits (set at the expanded uncertainty of the difference between result and certified value).

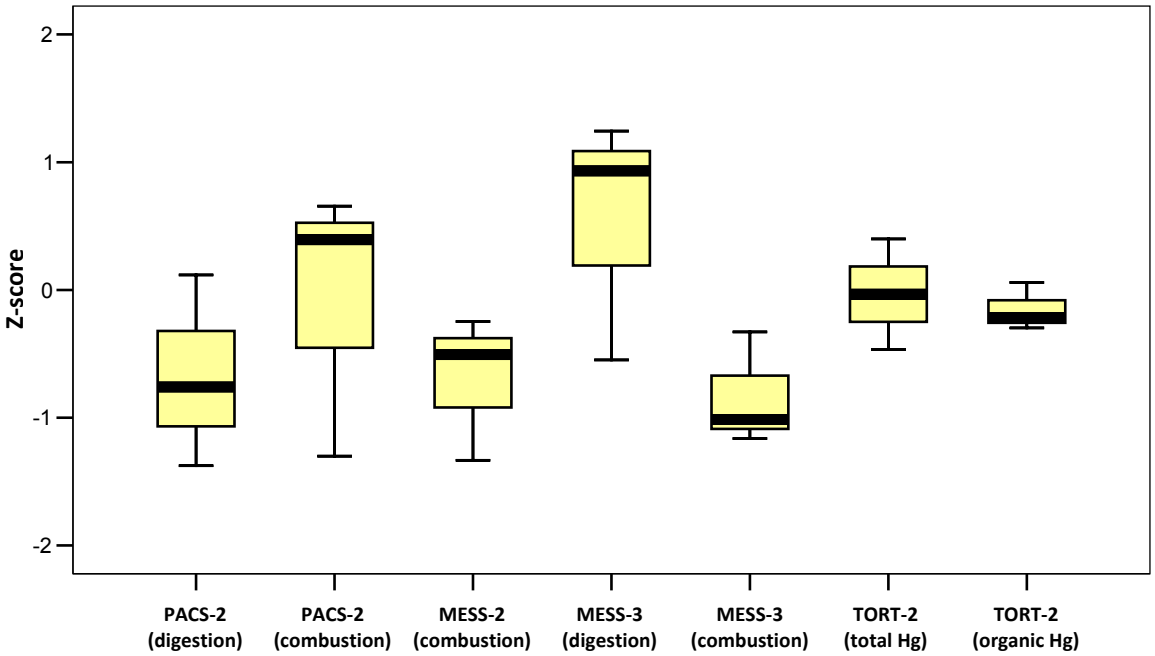


Figure 3.2 - Box plots of Z-scores for the main CRMs (median, interquartile range and range).

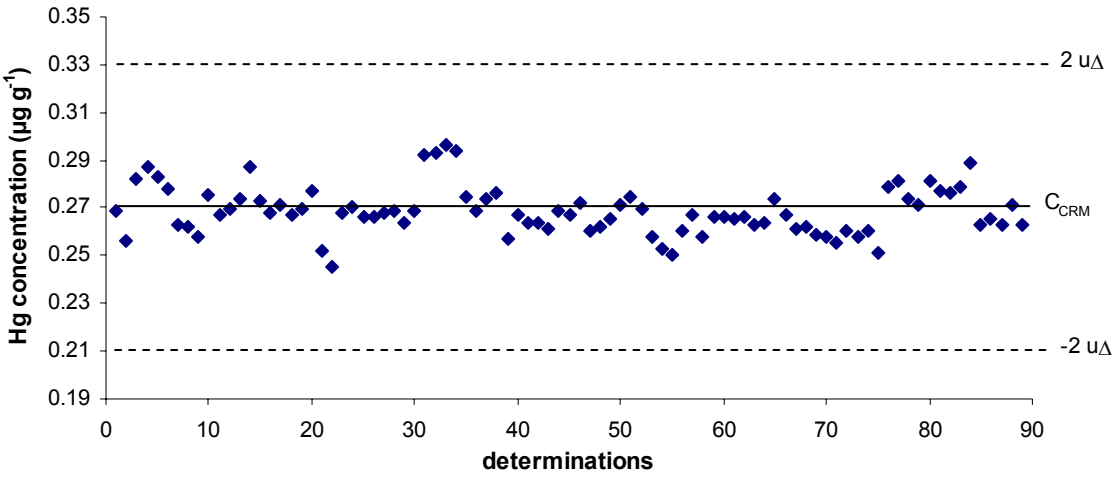


Figure 3.3 - Control chart of total mercury determinations in the certified reference material TORT-2.

The suggested bias observed for BCR-414 (all Z-scores were considered unsatisfactory) can be related to degradation of the material once the container was opened a long time before the use in the present work and therefore the correctness of the certified value cannot be guaranteed. These low recoveries were not used in corrections for recoveries, and the analysis of the BCR-414 was performed only for control of variability between batches of analysis.

In the determination of organic mercury, precision was always less than 10% for TORT-2 and 16% for IAIE-140TM.

Limits of detection

The range of variation of the MDL obtained for analytical methods involving mercury determinations are presented in Table 3.2. The presented limits of detection were estimated including all steps of the analysis. Replicate analyses of sample blanks were performed and the results and its uncertainty were taken into account.

Table 3.2 – Limits of detection for methods of mercury analysis.

Analysis	Technique	MDL
Reactive dissolved Hg	CV-AFS	0.6 – 1.2 ng L^{-1}
Total dissolved Hg	CV-AFS after photochemical digestion	0.9 – 2.8 ng L^{-1}
Hg in SPM	CV-AFS after digestion with HNO3	0.009 ± 0.057 µg g^{-1}
Hg in sediments	Electrothermal AAS	0.10 - 0.20 ng g^{-1}
Hg in biota	Electrothermal AAS	0.15 - 0.40 ng g^{-1}

In the quantifications involving the atomic fluorescence spectrometer, the LOD obtained through the calibration parameters and the one obtained by replicates analysis of procedural blanks were compared, and considering a defensive approach it was considered the higher of both as the minimum quantity that can be distinguished from the absence of the analyte. In fact, the MDL for the method of analysis of total dissolved mercury is higher than the MDL for reactive dissolved mercury, as a result of the higher manipulation of the samples necessary in the quantification of total mercury, which includes the uses of reagents.

4. Hg behaviour in the lagoon-ocean interface

4.1 Introduction

The distribution between suspended particles and water determines the relative concentrations of a chemical in each fraction, hence its reactivity, bioavailability, toxicity, transport and fate (Millward and Glegg, 1997; Le Roux et al., 2001; Leermakers et al., 2001; Turner et al., 2004). In dynamic zones such as estuaries and coastal zones, the understanding of mercury behaviour is for this reason essential in the evaluation of the input to the Ocean and its retention in the transitional waters. The high affinity of mercury for particulates and organic matter contributes in part to its environmental behaviour. In fact, the partition between particles and dissolved fraction determines the extension of trapping or dispersion of mercury in aquatic systems, making essential the understanding of processes and factors that control the partitioning (Johansson et al., 2001). The particle-water interactions of mercury in estuaries are largely determined by biogeochemical and sedimentological processes, with suspended particles playing a dominant role in the transport and scavenging of mercury (Coquery et al., 1997; Kim et al., 2004; Hissler and Probst, 2006). The association of mercury with solid fractions is responsible for the relatively low dissolved concentrations found in oxygenated natural waters (Tessier, 1992), making the study of the partitioning of mercury between particles in different size classes important. In addition, the effects of tidal regime and seasonal variability have been poorly investigated and may play an important role in the quantities of mercury exchanged between estuaries and their adjacent seas.

To understand the effects of physical and geochemical factors on the distribution of mercury in waters exchanging in the interface lagoon-Ocean, mercury partitioning and reactivity in the water column of the mouth of Ria de Aveiro were investigated during four tidal cycles in contrasting season and tidal regimes.

4.2 Sampling design

The cross section sampled was established at the outer boundary in order to evaluate both the properties and the exchanges between the lagoon and the Atlantic Ocean (Figure 1.1). To study the hydrodynamic homogeneity of the channel, a preliminary survey was conducted during a winter spring tide. In this pilot study, the transversal and vertical variability was studied with an acoustic current Doppler profiler to monitor the velocity and water flow in the transect between

the two margins. CTD (conductivity-temperature-depth) profiles (resolution of 50 cm) were performed in flood tide, ebb tide and slack water in three stations along the transect.

The plots of current velocities and of acoustic backscatter intensity (which is related to suspended solids concentration) in the sampled cross-section in the flood tide and ebb tide of the preliminary campaign are shown in Figure 4.1.

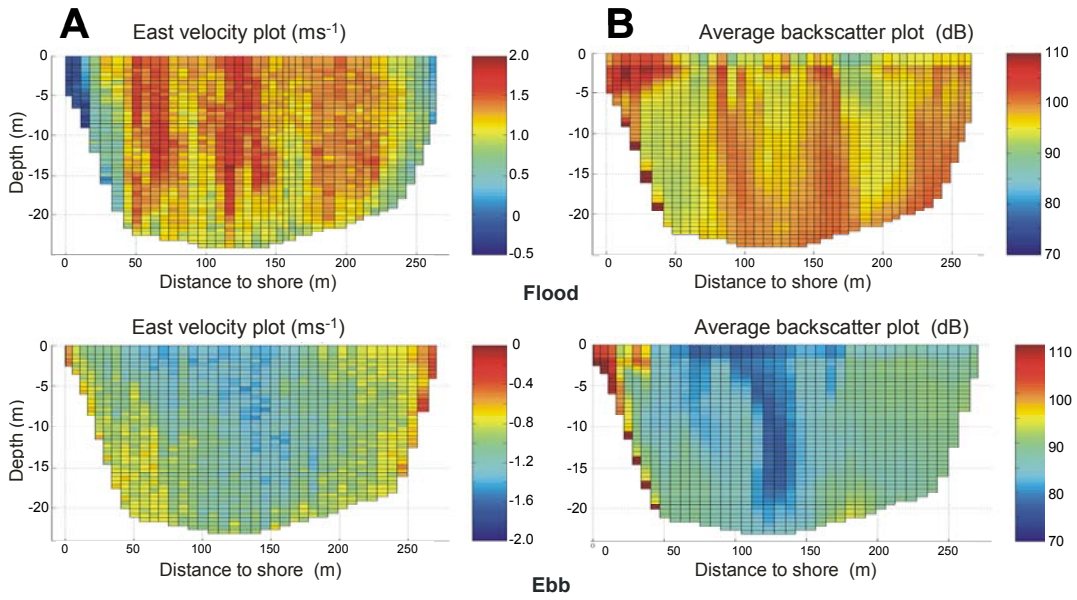


Figure 4.1 - A) Plot of current velocities (m s^{-1}) in the sampled cross-section during winter spring tide, B) Plot of acoustic backscatter intensity (dB) in the sampled cross-section during winter spring tide.

Although fairly homogeneous, some differences were observed in terms of transversal variability of currents, which were higher in the central zone of the cross-section. The acoustic backscatter intensity also shows some transversal variability as well as higher intensities near the bottom. Considering the turbidity and salinity profiles (Figure 4.2) in the preliminary campaign, small differences were observed between the three subdivisions of the cross-section.

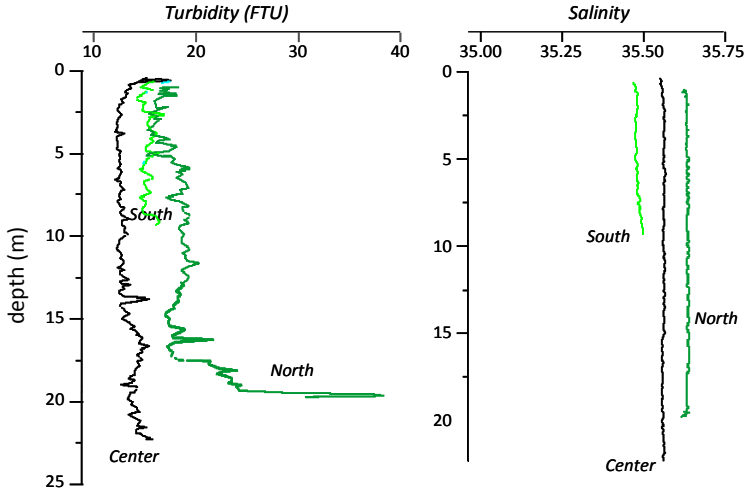


Figure 4.2 - Turbidity and salinity profiles in three subdivisions of the cross-section of the outlet channel.

In spite of the small variability observed in the water column, considering that sampling design should be defensive, it was decided to collect samples in three points along the cross-section (two close to each margin and one at the middle; coordinates shown in Table 4.1 in WGS 84 system) and at two depths (Figure 4.3).

Table 4.1 – Coordinates of sampling stations along the cross-section of the outlet-channel.

	Latitude	Longitude
North	40° 38' 46.9'' N	008° 44' 56.0 W
Center	40° 38' 43.3'' N	008° 44' 55.2 W
South	40° 38' 39.9'' N	008° 44' 55.3 W

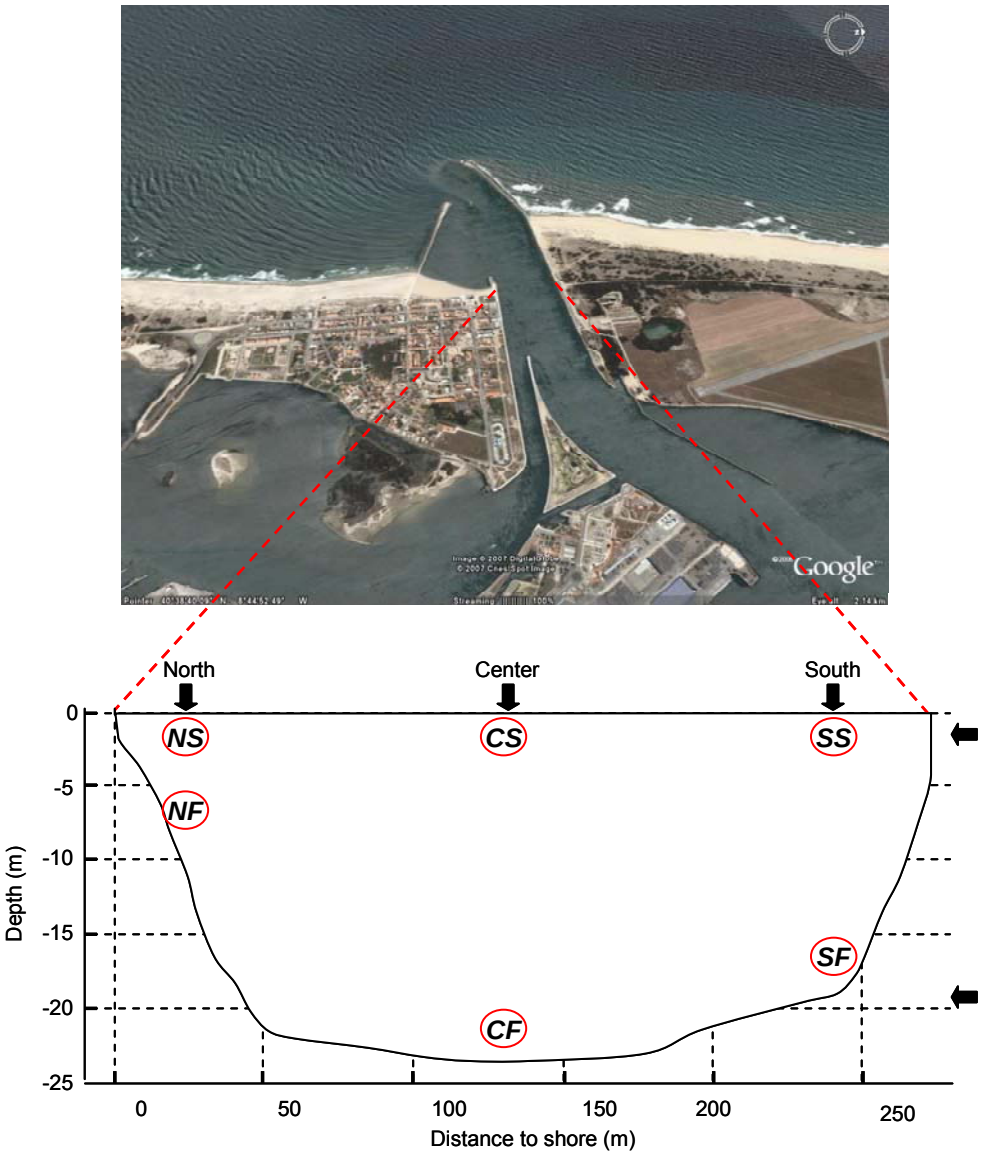


Figure 4.3 - Location of water sampling stations in the cross-section of the outlet channel.

To evaluate the seasonal and fortnightly variability of mercury behaviour, sampling was performed during tidal cycles in contrasting river discharges and tidal ranges. In winter, two campaigns were carried out, one during a neap tidal cycle (21st February 2002) with a tidal range of 1.24 m and another (28th February 2002) in a spring tidal cycle with a tidal range of 3.37 m. In summer, spring (26th June 2002) and neap (4th July 2002) tidal cycles sampled had tidal ranges of 1.06 and 2.81 m, respectively. To characterize the transport during ebb and flood tides, samplings were performed every 1.5 h in winter and every 3 h in summer, over semi-diurnal tidal cycles. In addition, surveys with an acoustic current Doppler profiler were performed at the time of the collection of water samples to monitor the velocity and water flow in the transect between the two margins.

4.3 Results and Discussion

4.3.1 Physico-chemical characteristics of the water column

Several physico-chemical properties are different in Oceans and in rivers, being expected that records during a tidal cycle will fluctuate in response to those differences of the two end-members and to the reactivity during the mixing.

The mean water temperature in winter at the outer boundary cross section was 13.6 ± 0.2 °C (mean ± standard deviation) at neap tide and 13.9 ± 0.1 °C at spring tide. During summer, differences between the maximum and the minimum temperature were more accentuated ranging from 14.7 ± 1.2 °C (neap tide) to 15.2 ± 0.7 °C (spring tide) (Table 4.2). The shallowness of the lagoon and the higher summer mean air temperatures explain the higher differences between the maximum and the minimum water temperature values in this season.

Table 4.2 - Ranges (maximum – minimum) of water temperature (T, °C) at the cross section during tidal cycles and along the vertical profile (depth in meters).

	Winter		Summer	
	Neap tide	Spring tide	Spring tide	Neap tide
Depth	T, °C	T, °C	T, °C	T, °C
2.0	13.2 - 13.9	13.6 - 14.0	14.5 - 16.3	13.6 - 17.8
10.9	13.4 - 13.9	13.7 - 14.0	14.4 - 16.24	13.6 - 16.4
19.8	13.4 - 13.9	13.7 - 14.0	14.6 - 15.8	13.6 - 16.2

Seasonal and tidal variations of salinity during tidal cycles are shown in Figure 4.4A. To simplify the graphic representation and because the two depths have different sampling frequencies in winter, only the surface values are connected by a line. The salinity follows the tidal cycle pattern, increasing during the flood and with minimum at low tide. The higher salinity range was observed in winter (rainy season) as a result of the higher freshwater discharge, especially during neap tide (mean of 33.2 and salinities ranging from 29.0 to 35.0). In winter spring tide, the mean salinity was 33.5, ranging between 30.8 and 34.5. In summer, lower salinity ranges were observed (from 33.9 to 34.8 in spring tide and between 33.0 and 35.5 in neap tide). The mean salinity at spring tide was 34.4 and the mean salinity at neap tide was 34.6. These narrow ranges of salinities evidence a substantial marine influence in the inlet channel in contrasting seasonal and tidal range conditions.

Salinity at three depths (2, 11 and 20 meters) during the tidal cycles was compared by a paired t-test. No significant differences (p-level of 0.05) were found, except for winter at neap tide between conditions at 2 and 11 m depth.

Considering the pH (Figure 4.4B), the values also followed the tidal cycle as a result of the mixture of the two end-members with different pH. Seawater is highly buffered and slightly alkaline, which resulted in increasing pH during flood tide. The buffering is controlled by carbonate and borate equilibriums, resulting in pH usually around 8.1 and 8.5. The higher humic content at the low salinity end-member results in lower pH, with values between 6.5 and 7.5 (Ramalhosa, 2002).

Fluctuations of suspended particulate matter concentrations are assumed to be essentially a function of current velocity and of the result of erosion/deposition processes (some induced also by the wind). Concentrations of SPM (Figure 4.4C) were comparatively higher (Mann-Whitney Rank Sum Test; $p<0.001$) during winter, with SPM concentrations ranging in winter from 11.7 to 90.1 mg L^{-1} and in summer between 9.5 and 39.6 mg L^{-1} as a result of higher fresh water input (rivers and land drainage), in addition to wave action and wind climate. In addition, during neap tides, wind blew with intensities (>20 km h^{-1}) higher than the ones observed during spring tides, which have certainly contributed to comparable SPM concentrations in tidal cycles of the same season but different tidal ranges. For this reason, in spite of the fact that tidal currents during spring tides have double intensity of neap tide currents (Dias et al., 2003), the resulting erosion sometimes was not easily distinguished from the wind-induced effects. This wind-induced turbulence is able to erode fine bed sediments in the large shallow areas and in intertidal zones. Additionally, under tidal influences, the current strength controlled the advective transport of

SPM (in both directions) as well as the vertical transport (deposition and resuspension). The periods of maximum current speed in the channel (mid-ebb and mid-flood) were not always coincident with the highest levels of SPM. These phase shifts are reasonable considering the time delay between the maximum currents in the shallower zones and the time for lateral advection until transport to the sampling station in the outer boundary. In addition, the already mentioned resuspension related to wind-induced turbulence can also contribute to changes in the pattern of suspended particles during the tidal cycles. The occurrence of resuspension is also evident by the higher SPM levels in the bottom of the water column (especially in the winter neap tide; Mann-Whitney Rank Sum Test; $p<0.001$).

Concerning the SPM concentrations observed in the winter neap tidal cycle (considered high in comparison with the corresponding spring tidal cycle), they are in agreement with the results reported for the same sampling station a day before the sampling of the present study, and were attributed to high coastal wave energetic conditions (Abrantes et al., 2006). In fact, measurements from the nearer wave buoy (located in Leixões, Porto) indicated that significant wave heights exceeded 3 meters with average periods of 7-8 s. These conditions might also have contributed to the increase of SPM levels during the winter neap tidal cycle as a result of resuspension of fine fraction of bed sediments in the continental slope, followed by supplying to the lagoon by the flood currents. Concluding, the SPM concentrations were found to be a result of a complex interaction of several factors that increased the bed-shear stress, with an important role played by tidal currents, wave energy and wind (Lopes et al., 2006b).

The particulate organic carbon in the water column of estuaries are the result of carbon of living organisms (mainly phytoplankton, as well as bacteria and animals) and non-living particles (detritus, i.e. all types of biogenic material in various stages of decomposition, which represent potential energy sources for consumer species). In the tidal cycles, particulate organic carbon content varied between 0.98% and 7.21% (Figure 4.5A) and was significantly higher in spring tides (Mann-Whitney Rank Sum Test; $p<0.001$). In spite of the fact that the overall median of POC in the summer (3.9%) was higher (Mann-Whitney Rank Sum Test; $p<0.001$) than the overall winter median (3.0%), the intra-tidal variability was more pronounced than the seasonal and fortnightly.

Throughout the sampling period, DOC varied between 0.7 mg L^{-1} and 2.5 mg L^{-1} (Figure 4.5B), with no significant variation between seasons (Mann-Whitney Rank Sum Test; $p=0.702$) and between tides (Mann-Whitney Rank Sum Test; $p=0.053$). The overall mean ($n=144$) of DOC determinations was 1.5 mg L^{-1} with a standard deviation of 0.3 mg L^{-1}. Only in winter did the

concentrations seem to follow the tide. In general, no clear trend was observed for concentrations over the tidal cycles (only in winter a small increase is observed during the ebb tide), which is reasonable, bearing in mind the marine influence noticed during the campaigns and that DOC levels in the upper estuary are only slightly higher, ranging generally between 1.5 and 4 mg L^{-1} (Ramalhosa, 2002; Monterroso, 2005). The muddy sediments found in the shallower zones of the lagoon have pore-waters enriched in organic matter (Monterroso, 2005), which can act as a source of DOC to the overlying water column.

The DOC concentrations in waters in the outer boundary of the Ria de Aveiro, can be considered similar to levels observed in other estuaries (Laurier et al., 2003), but are low when compared with levels in other estuarine systems (Stordal et al., 1996; Lacerda and Gonçaves, 2001; Turner et al., 2002).

Phytoplankton chlorophyll a (Chl a) concentrations may be used as an indicator for the abundance of these primary producers. In addition to the ability to bioaccumulate mercury, phytoplankton has a preferential uptake or sorption of methylmercury compared with inorganic mercury (Stoichev et al., 2006b) and therefore contributes to the mobility of toxic contaminants within the environment or through the trophic web. It is recognised that chlorophyll concentrations are a function of season and have a minimum in winter months. Accordingly, results over the tidal cycles show that the Chlorophyll *a* concentrations (Figure 4.5C) were much lower during winter (Mann-Whitney Rank Sum Test; $p<0.001$), as expected. In winter, concentrations ranged between 0.90 and 4.93 µg L^{-1} Chl *a*, with medians of 1.78 µg $L^{-1}$and 2.10 µg L^{-1} in neap tide and spring tide, respectively. The highest primary production and the highest range of Chl *a* concentrations in a tidal cycle were recorded during summer neap tide with a median of 6.92 µg L^{-1} and concentrations ranging from a minimum of 1.98 µg L^{-1} at low tide, to a maximum of 17.7 µg L^{-1}. The coastal zone of the Ria de Aveiro belongs to the Northeast Atlantic Upwelling Region, where predominant trade winds from the north cause persistent upwelling conditions prevailing from June to September (Smyth et al., 2001; Relvas et al., 2007). This upwelling of nutrient-rich waters is efficiently used by the phytoplankton during the summer months promoting high levels of phytoplankton and zooplankton production.

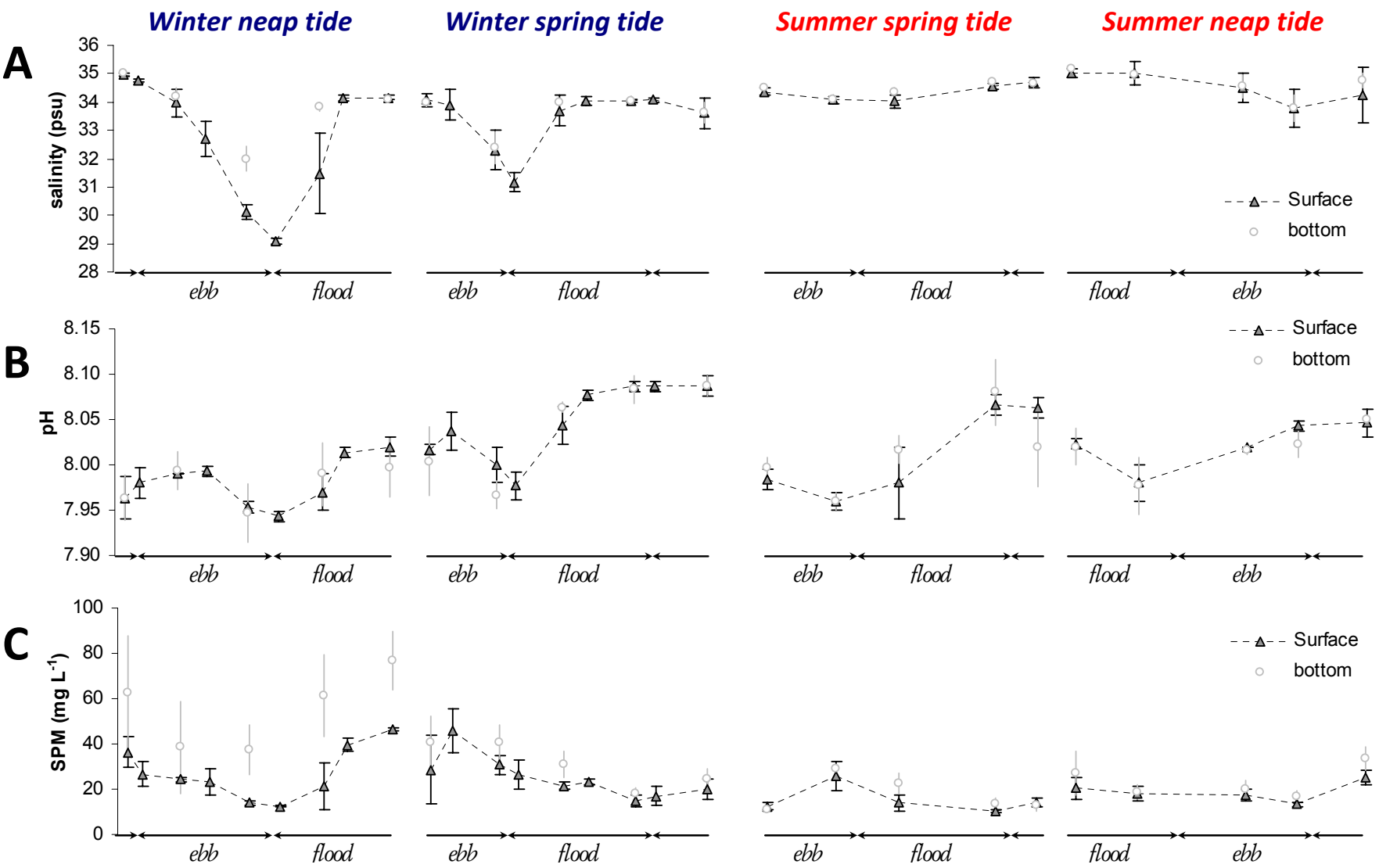


Figure 4.4 - Seasonal and tidal variation of salinity (A), pH (B) and SPM (C) over tidal cycles (mean values and standard deviation).

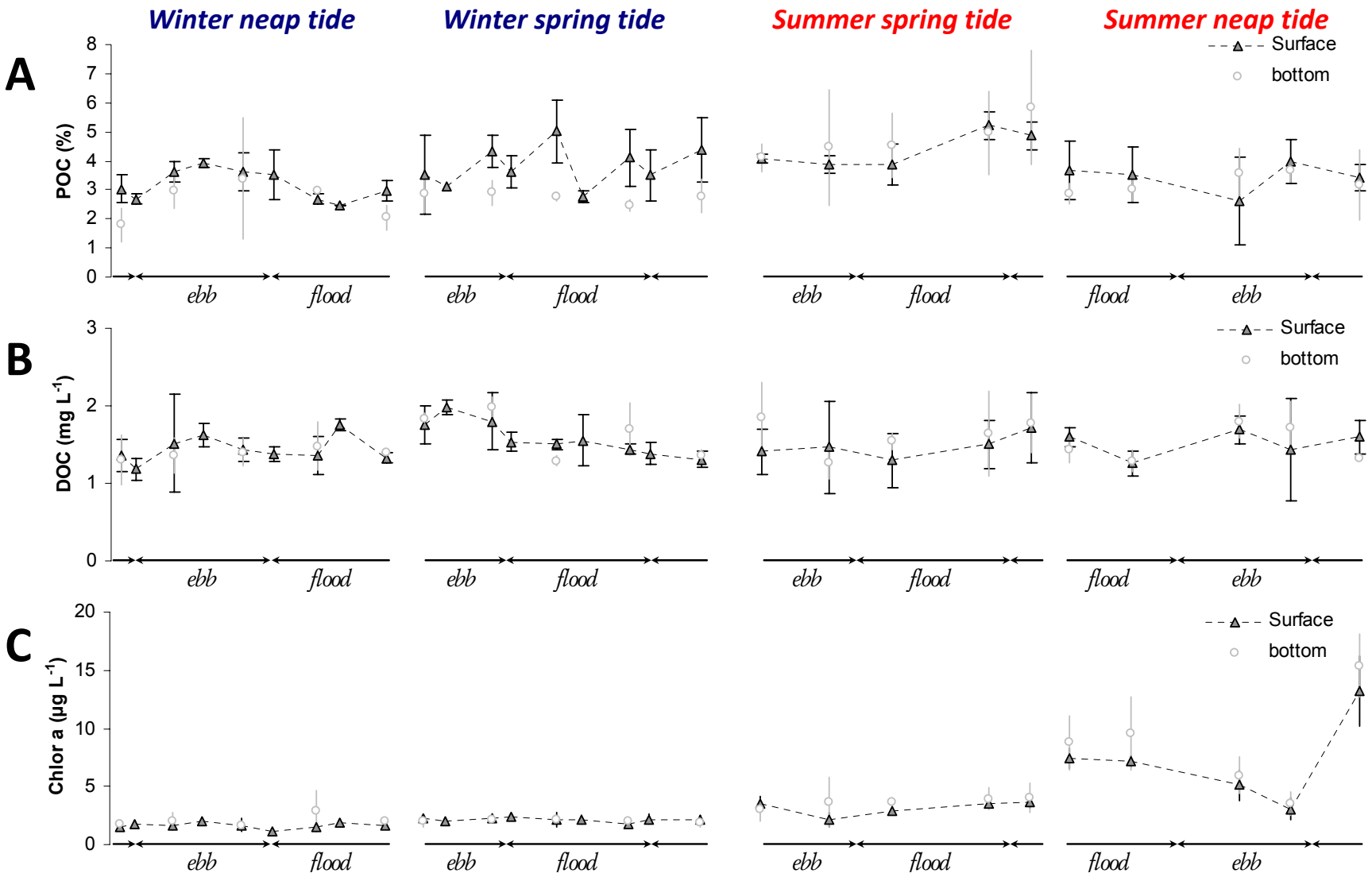


Figure 4.5 - Seasonal and tidal variation of POC (A), DOC (B) and Chlorophyll a (C) over tidal cycles (mean values and standard deviation).

Seston may be subdivided into living (phytoplankton, bacteria, protozoa, zooplankton) and non-living (detritus) fractions. The simple observation of the samples collected with the 63 µm and the 200 µm mesh size plankton nets evidenced the presence, in some of them, of a significant fraction of sediment particles. In fact, the concentrations of seston material followed a similar pattern to the SPM (with very significant correlations in some campaigns; $p<0.001$), suggesting a significant contribution of detrital particles to the composition of the seston (Figure 4.6). In winter neap tide, seston concentrations increased during the flood tide, probably was a result of the already mentioned wave energetic conditions observed in the coastal zone. Maximum concentrations at the surface were recorded at winter spring tide (2960 μgL^{-1}) during ebb tide.

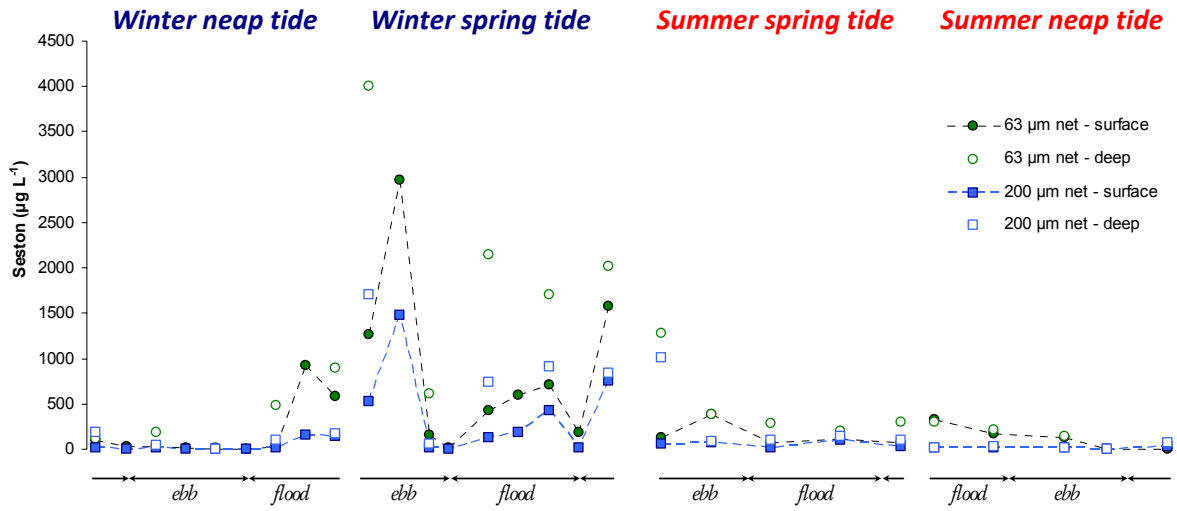


Figure 4.6 - Seasonal and tidal variation of seston concentrations collected with 63 µm and 200 µm nets over tidal cycles.

The tidal phase was found to influence not only the amount of suspended material, but also its composition. Tidal variation in seston concentrations and composition has been observed in other estuaries (Prins et al., 1996; Huang et al., 2003), and the carbon contents of both plankton nets reveal this distinct composition (Figure 4.7). The high variability along the tidal cycles reflects the already mentioned occurrence of sediment resuspension. In winter spring tide, low carbon concentrations were observed in several samples (*circa* 0.3%), with values one order of magnitude lower than the ones observed in neap tide. In neap tides, the carbon content in the plankton nets was negatively correlated with the amount of SPM (Pearson's Correlation, $p<0.05$), corroborating the influence of resuspension on the composition of collected material. Also, the carbon content of the 200 µm net material was statistically higher (Mann-Whitney Rank Sum Test, $p<0.001$) than the 63 µm net material, suggesting a higher biogenic content in 200 µm seston which probably is a result of the small grain size of resuspended material. The presence of

resuspended particles is also evident in the lower carbon content of the seston collected in the bottom of the water column.

Considering the influence of the tidal phase in the composition of seston, at high hydrodynamic periods of the tide (high turbidity) the lithogenic fraction of seston increased, which resulted in a decrease of the biogenic fraction (reflected by a decline in a biogenic tracer such as carbon content). On the contrary, maxima levels of carbon content were observed generally in periods of lower currents (near slack water) and therefore when resuspension of lithogenic material is minimal (Monterroso et al., 2003). As a matter of fact, a significant decrease ($p<0.001$ in both nets) of carbon content in seston was observed with increasing seston concentrations in the water column. Especially clear in the winter spring tide, is the presence of lithogenic material in the material collected in the nets in periods of higher currents.

Taking into account that biogenic and lithogenic particles differ in their affinity and behaviour towards pollutants, the knowledge of the partition between biogenic and lithogenic material in seston is essential for researchers focused on processes involving metal bioavailability and bioaccumulation in plankton communities.

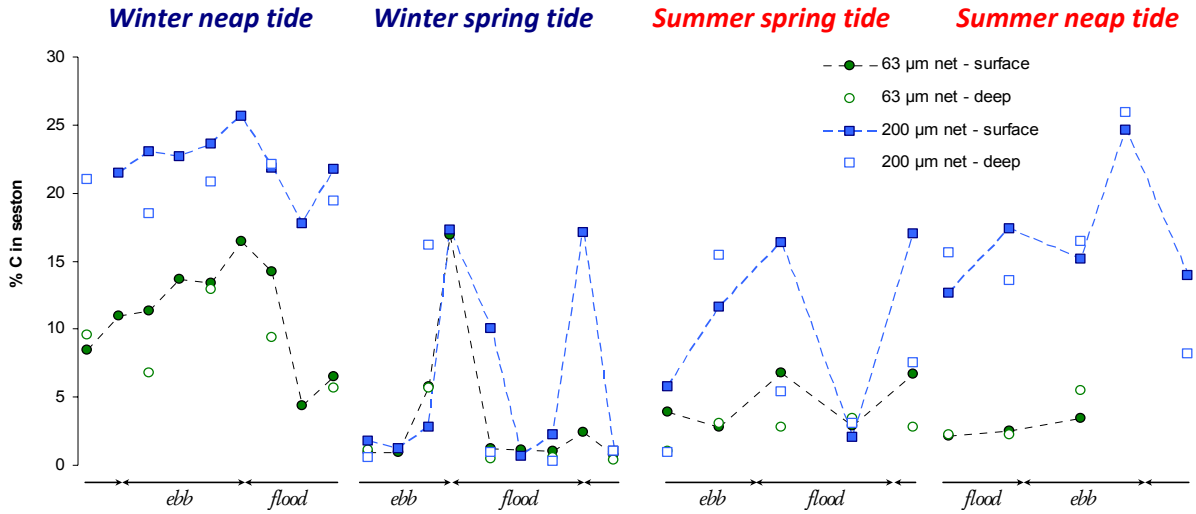


Figure 4.7 - Seasonal and tidal variation of carbon content (%) in seston collected with 63 µm and 200 µm nets over tidal cycles.

Tidal resuspension can therefore be considered one of the most important forcing functions regulating the composition of seston and the partition of suspended biogenic and lithogenic particles in mesotidal estuaries. In addition, other factors promoting resuspension such as wind driven turbulence, dredging activities and trawl fishing should be taken into consideration in the course of sampling and results evaluation. There are obvious implications from these observations

for planktonic research, since most studies in this area dealing with contaminants usually consist of seston collection and chemical analysis of the bulk net material.

The ratio of carbon to nitrogen in organic matter has been used to differentiate source material as well as to characterize seston quality (Faganeli et al., 1988; Meyers, 1997; Lamb et al., 2006; McConnachie and Petticrew, 2006). In spite of the large limitations of C/N ratio to indicate definitely the origin of organic matter, the variability of the ratio during the tidal cycles corroborates the variation of seston composition (Figure 4.8). This indicator corroborates the enrichment of material of lithogenic origin during winter, with C/N ratios around 15 (Graham et al., 2001) on some occasions, as already suggested by the carbon content of seston. In periods of slack water, the reduced resuspension contributed to an enrichment of the biogenic fraction of seston (4 < C/N ratio < 8) (Meyers, 1997).

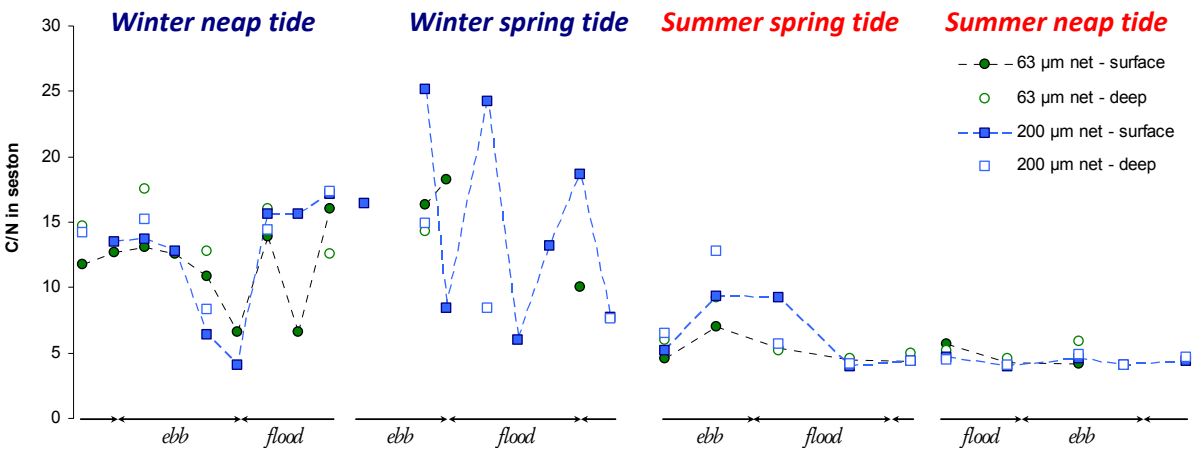


Figure 4.8 - Seasonal and tidal variation of carbon:nitrogen ratio in seston collected with 63 µm and 200 µm nets over tidal cycles.

The aluminium content of seston would confirm this observation, as this element is a good tracer of terrestrial influence (Monterroso et al., 2003). Hence, it is evident that C/N data is limited to establishing precise statements about the nature of the material and the source of organic matter, and that further characterization would be necessary, e.g., measurements of stable isotopes of carbon and nitrogen (Meyers, 1997; Lamb et al., 2006; McConnachie and Petticrew, 2006), and the already mentioned, aluminium content.

4.3.2 Relationships between master variables

The study of relationships between variables is an important tool for the characterization of both particulate and dissolved fractions of the water column.

In Figure 4.9 the relationship between pH and salinity is presented. As a result of the higher pH of seawater, the pH showed an increase with salinity in the surveys with the higher salinity range.

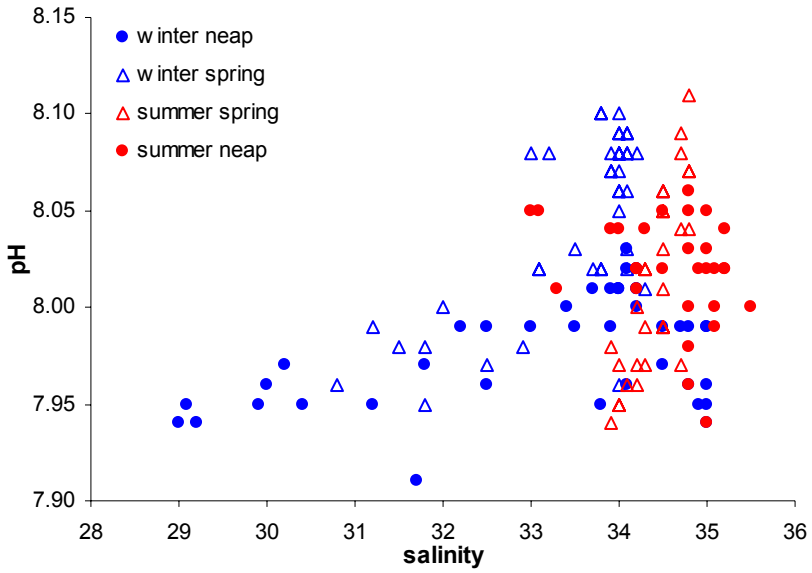


Figure 4.9 – Relationship between salinity and pH in the tidal cycles.

The relationship of salinity and SPM is shown in Figure 4.10. Although higher SPM levels are noticed for high salinities, no general trend is observed. Moreover, these higher SPM concentrations (above 55 mg L^{-1}) were observed for winter neap tide and are all related to bottom waters entering the lagoon during the flood tide. For this tidal cycle, some of the lower SPM concentrations were found at the slack water period at the end of the ebb tide, and therefore linked with the lowest salinities. Only for summer spring tide a decrease (spearman coefficient = -0.602; $p<0.001$) of SPM was observed together with the increase of salinity, due to lower SPM load in flood tide waters.

Considering the relationship between salinity and POC (Figure 4.11), only in the winter neap tide a significant decrease of POC was observed with increasing salinity (Spearman coefficient=-0.436; $p=0.004$) which denotes an enrichment in organic carbon of the fluvial particles.

No relationship was observed between DOC concentrations (Figure 4.12) and salinity because the DOC levels in the two end-members are similar and the salinity range relatively narrow.

As observed for seston, also the composition of SPM reflected the dynamic of sediment resuspension. The relationship between POC (%) and SPM concentrations (Figure 4.13) evidence a clear decrease ($p<0.05$) of the organic content of the particulate with increasing SPM concentrations. This means that the high SPM concentrations observed in the bottom waters

during the winter neap tidal cycle are to some extent the result of resuspension of coarser sediments with lower organic content than the permanently suspended particles, usually travelling through the estuary. The carbon depletion of particles travelling in bottom waters seems to be higher during the flood tide, which is in agreement with the presumption of resuspension of particles in the marine coastal zone. Considering the distribution of POC content and SPM load during the tidal cycles, the exit during ebb tide of particles that entered the lagoon in the previous flood tide and vice-versa (i.e., the transit through the inlet of some particles resuspended both inside and outside the lagoon) cannot be disregarded.

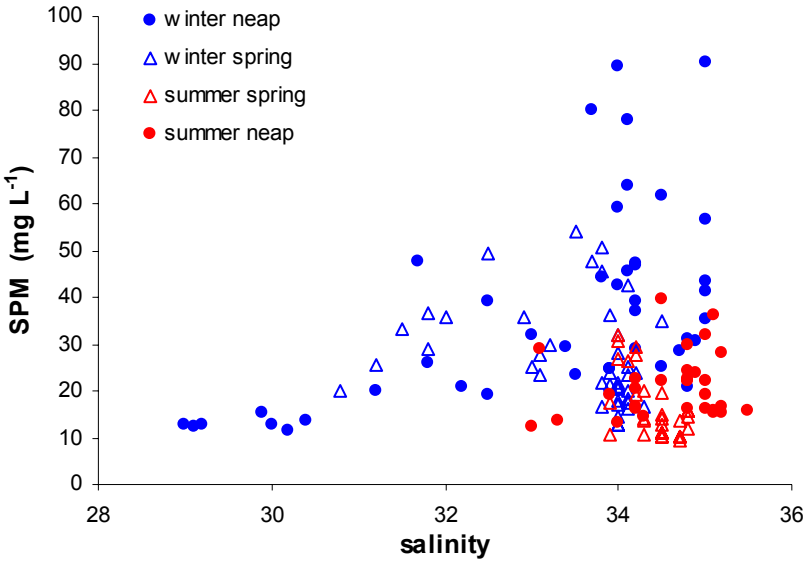


Figure 4.10 - Relationship between salinity and SPM in the tidal cycles.

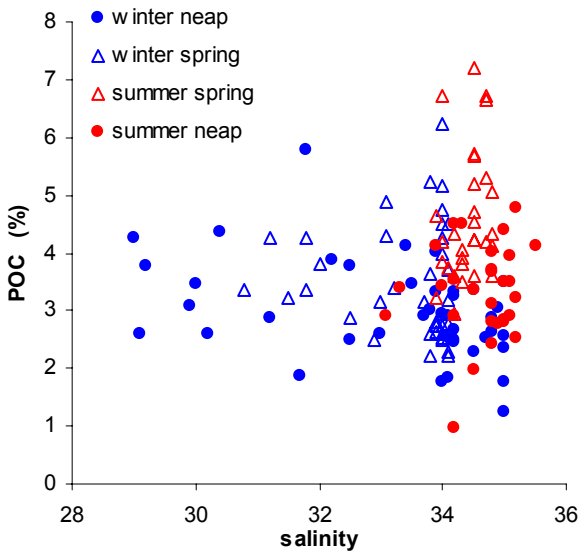


Figure 4.11 - Relationship between salinity and POC (%) in the tidal cycles.

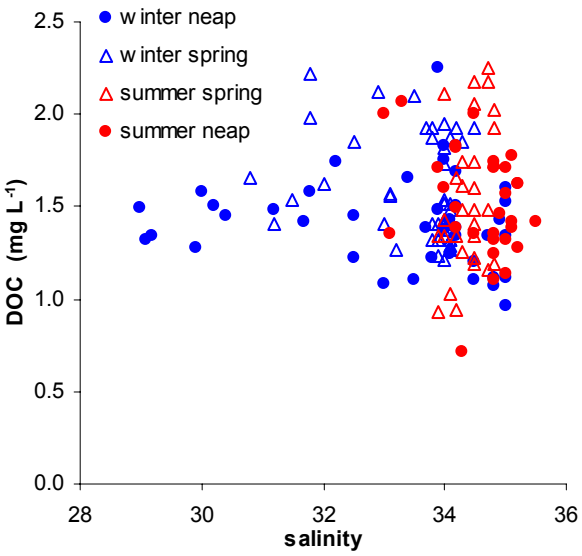


Figure 4.12 - Relationship between salinity and DOC in the tidal cycles.

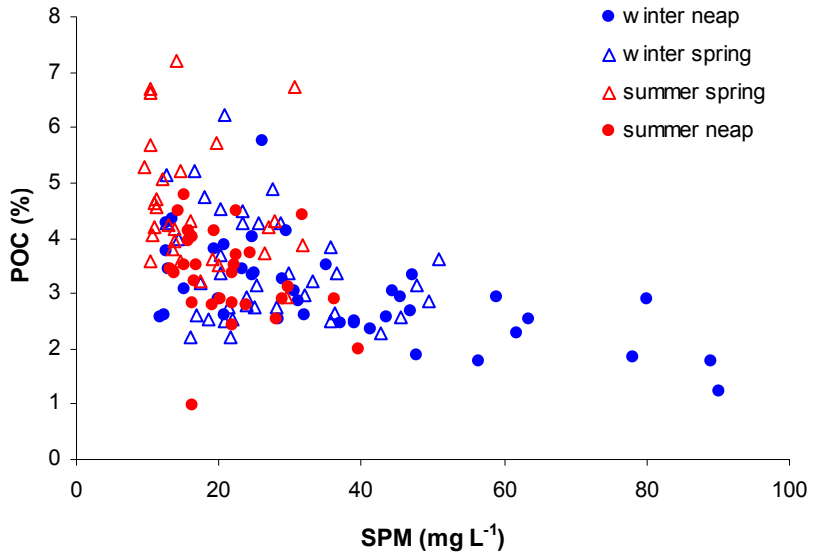


Figure 4.13 - Relationship between SPM and POC (%) in the tidal cycles.

For chlorophyll a, only in the summer spring tide an increase with salinity (Figure 4.14) was observed ($p<0.05$), evidencing that the Ocean was a source of phytoplankton to the lagoon. Nevertheless, an enrichment of pigment in particulate matter (weight/weight; Figure 4.15) was observed in the summer with increasing salinity ($p<0.05$). On the contrary, a decrease was observed in the winter neap tide ($p<0.01$). Thus, the particulate phase in summer has a higher phytoplankton content in marine particles, while in winter the marine particles seem to be have a lower phytoplankton fraction.

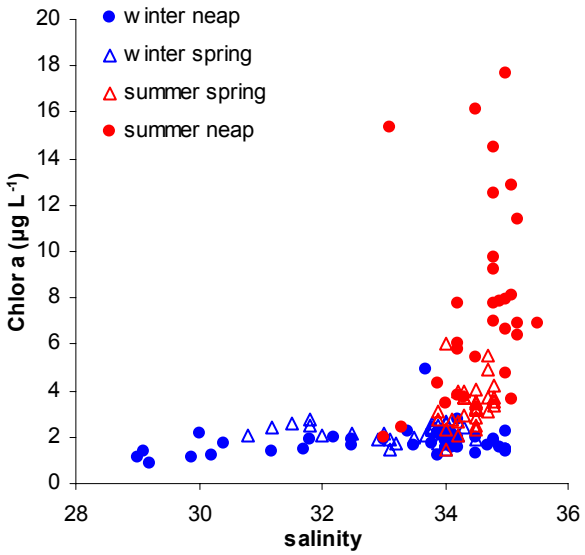


Figure 4.14 - Relationship between salinity and Chlorophyll *a* in the tidal cycles.

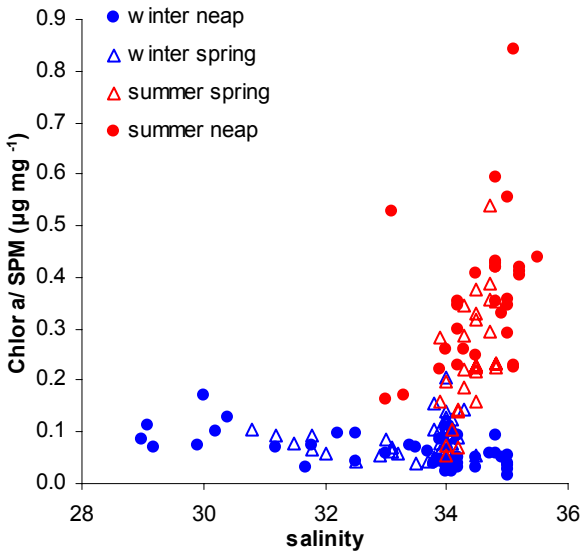


Figure 4.15 - Relationship between salinity and Chlorophyll *a* per mass of SPM in the tidal cycles.

4.3.3 Mercury in the water column (dissolved and particulate fractions)

Unlikely some simpler constituents or properties of water, the behaviour of mercury during mixing is very complex, and the variations along the tidal cycle are expected to be largely influenced by the reactivity of mercury (Coquery et al., 1997; Le Roux et al., 2001; Rolfhus et al., 2003; Stoichev et al., 2006b).

Reactive dissolved mercury concentrations in the four tidal cycles are presented in Figure 4.16A. Levels were higher during winter (maximum of 5.8 ng L^{-1}), and during summer most of the values were below the limit of detection (which ranged between 0.6 and 1.2 ng L^{-1}; in Figure 4.16A the limit of detection was shown as 1 ng L^{-1}). Still, winter levels were not very high, with 80% of detectable concentrations below 2.3 ng L^{-1}. During the winter tidal cycles the reactive dissolved mercury concentrations followed to some extent the salinity trend, denoting the influence of competing ions in seawater and the formation of chloro-complexes (also noticed in the behaviour of the fraction of reactive dissolved mercury species; representation not shown).

In the winter campaigns the fraction of reactive dissolved mercury was very variable. In winter neap tide the 10^{th} and 90^{th} percentiles of the percentage of mercury present in reactive forms were respectively 17% and 67%, and in the winter spring tide 8% and 45% for the same percentiles. The low concentrations of DOC contribute to the occurrence of some high fractions of reactive species. In contrast, the presence of colloids can be responsible for reducing the fraction of reactive mercury in the filter passing portion (Guentzel et al., 1996; Choe et al., 2003.

Total dissolved mercury (Figure 4.16B) displayed a high variability, ranging from 1.5 ng L^{-1} to 26.6 ng L^{-1}, and with no clear pattern over the tidal cycles. The variations during the tidal cycles were more pronounced than seasonal and fortnightly variability. The overall median in all surveys (n=143) was 6.6 ng L^{-1} and 88% of the results were lower than 10 ng L^{-1}. The higher total dissolved mercury levels observed during the winter spring tide are probably related to the higher tidal currents which induce resuspension of particles in the shallow and, generally, more contaminated areas. Moreover, addition to the dissolved fraction is also enhanced by salt intrusion that moves more upstream in spring tides, with an increase in particle-water exchange.

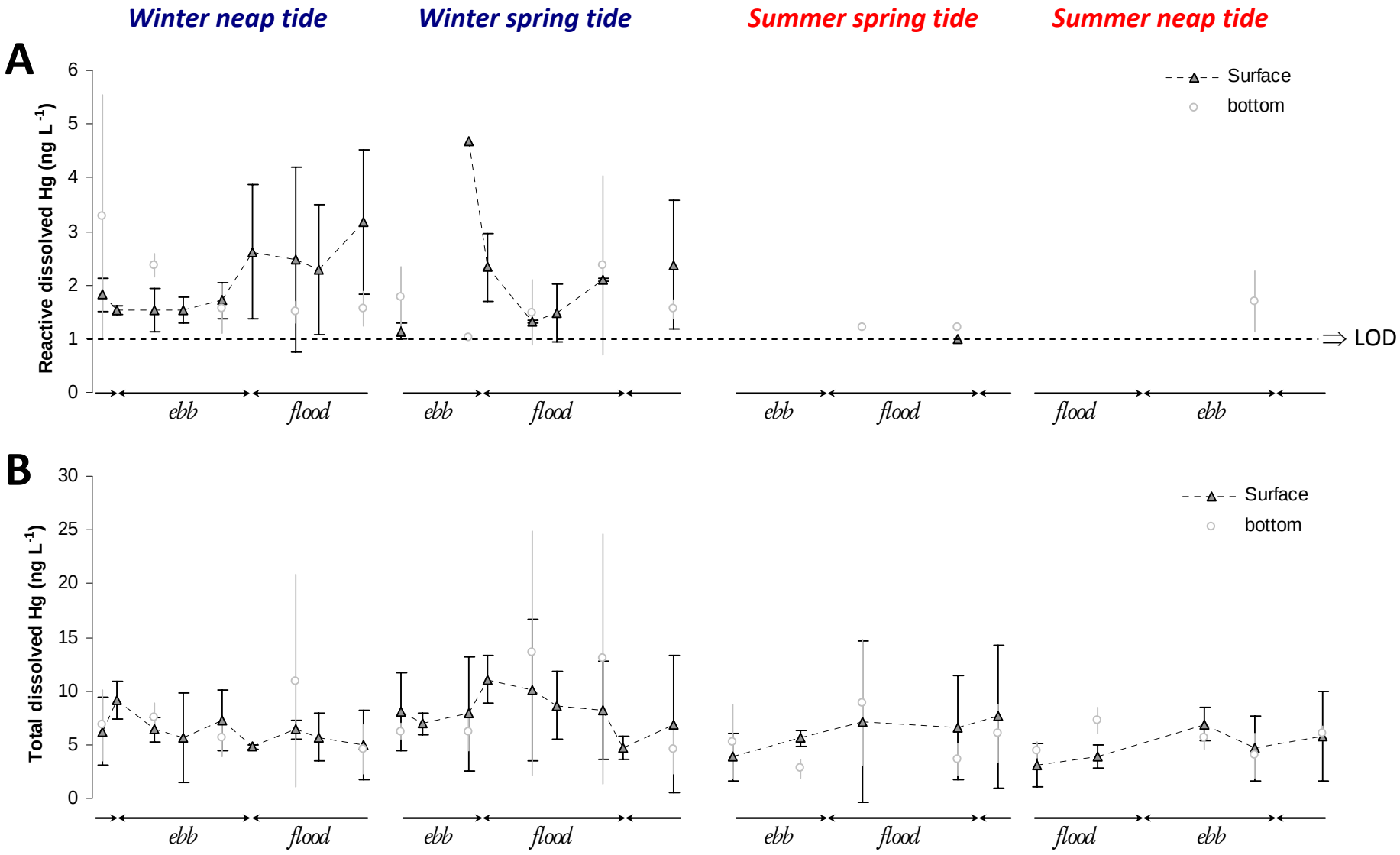


Figure 4.16 - Seasonal and tidal variation of reactive dissolved Hg (A) and total dissolved Hg (B) during tidal cycles (mean values and standard deviation).

Regarding the total mercury in SPM (Figure 4.17), seasonal variations were clearer than the fortnightly variations. Figure 4.17A shows the amount of mercury per mass of SPM (weight/weight) with levels ranging between 0.23 μg g^{-1} and 1.05 μg g^{-1} (overall average of 0.51 ± 0.03; 95% confidence interval, n=144). The higher concentrations were observed during the winter neap tidal cycle (the campaign where occurrence of wind-resuspension was identified by SPM load and carbon content in SPM), and were significantly different from levels in the other winter campaign and from the other neap tidal cycle (Mann-Whitney Rank Sum Test; $p<0.001$). During spring tidal cycles, the mercury concentrations in SPM were different in surface and bottom waters: in the winter spring tidal cycle, surface SPM showed higher concentrations of mercury (paired t-test; $p=0.006$), whereas in the summer tidal cycle of higher tidal range, the higher mercury levels in SPM were observed in the bottom samples (paired t-test; $p=0.019$). The higher mercury concentrations in SPM were observed during the ebb of the windy winter neap campaign, which corroborates the importance of winds in the increase of bed-shear stress through wave-induced sediment stirring (Paphitis and Collins, 2005).

The volumetric expression of particulate mercury levels (mass of mercury in SPM per volume of water) are very important when considering the study of the transport of mercury associated with movement of water masses, as presented in Figure 4.17B. In the winter neap tide, particulate mercury volumetric concentrations were much higher in bottom waters (paired t-test; $p = 0.002$), as a result of the higher load of suspended particles. The comparison of the SPM levels and concentration of particulate mercury (mass/volume) during each tidal cycle evidences the key importance of turbidity in the definite particulate mercury load in the water column.

Up to now, increases of both dissolved and particulate mercury have been attributed to the mobilization of mercury associated with bottom sediments. In fact, it is important to recall that bed sediments are net sinks for mercury, due to the binding of mercury to particles with organic coatings and/or precipitated as sulphides (Kim et al., 2004; Ramalhosa et al., 2006b). In the Laranjo basin, the mercury in surface sediments seems to be associated both with organic matter and with iron oxides (Ramalhosa et al., 2006b) and with sulphides in anoxic conditions (Ramalhosa et al., 2006a). However, some of this mercury can be remobilized by sulphide oxidation or complexation with dissolved organic ligands (Mason et al., 2006). Moreover, because of perturbations at the sediment/water interface (i.e. tidal and wind stirring, freshwater flows and bioturbation) contaminated sediments can be resuspended and dispersed within the water column (Pereira et al., 1998b; Kim et al., 2004; Rajar et al., 2004).

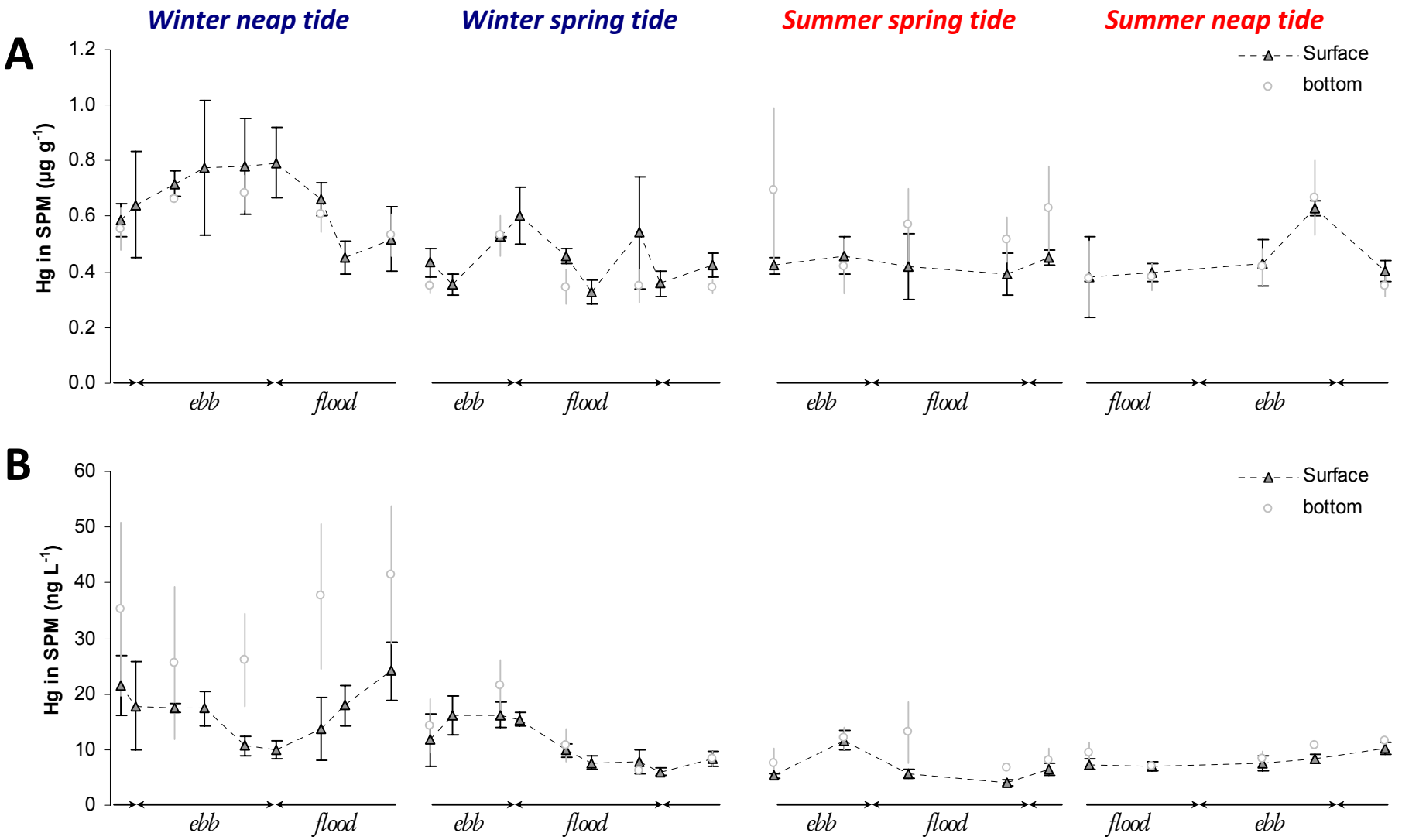


Figure 4.17 - Seasonal and tidal variation of total particulate Hg (weight / weight – A; weight/volume - B) during tidal cycles (means and standard deviations).

In fact, during winter the hydrology is significantly affected by these factors, contributing to an enhanced mobilization of mercury. These results are in agreement with Kim et al. (2004) where significant amounts of mercury were released into the water column, and also with the results from the Gulf of Trieste where most of the mercury is transported in particulate form (Rajar et al., 1997; 2004). Schäfer et al. (2006) also highlighted the remobilization of mercury in historically contaminated sediments due to riverbed dredging and flood-related sediment erosion. The presence of maxima percentages of methylmercury (Ramalhosa, 2002) and maxima methylmercury levels (Muresan et al., 2007) in superficial sediments means that increasing pressures over the sediment-water interface will result in a enhanced input of methylated mercury species to the water column and consequently an aggravation of mercury bioaccumulation and biomagnification.

Mercury concentrations in seston during the tidal cycles are shown in Figure 4.18. The observed variability of mercury concentrations are to a great extent a result of the variability of the nature of the material retained in the nets. The tidal effect was similar in the levels of mercury in both seston and SPM: in ebb conditions the levels of mercury have an increasing trend, while in flood conditions the levels of mercury tend to decrease. The highest levels were observed during winter and, in general, concentrations increased during the ebb tide. In the winter spring tide survey, the maximum mercury concentrations observed at the end of the ebb tide were 30 times higher than the minima levels at the end of the flood tide. Concentrations in seston collected in the 200 µm net were generally higher denoting some biomagnification of mercury through planktonic food webs. All these fluctuations of mercury levels in seston influence the transport of the metal. The concentrations of mercury in seston were one to two orders of magnitude lower than the mercury levels observed near the contaminated area (Monterroso et al., 2003).

As for SPM, the evaluation of the transport associated with seston, in waters exchanging between the lagoon and the Ocean, have to be considered in terms of concentrations per volume of water. Thus, the volumetric concentrations of mercury in seston along the tidal cycles are presented in Figure 4.19. The higher concentrations were observed during winter in the spring tidal cycle. As for SPM, the comparison of the pattern of seston load in the water column with the volumetric concentrations of mercury in seston, reveal the huge importance of the particles abundance to the amount of particulate mercury per volume.

Considering the three mercury fractions (dissolved, SPM and seston), the highest volumetric concentrations were observed for the total mercury in SPM (ranging from 9 to 55.3 ng L^{-1} in the winter neap tide), while the maximum value for total mercury in seston (0.13 ng L^{-1}) was 1-2

orders of magnitude lower than the average value for total dissolved mercury and for total mercury in SPM. The abundance of seston in the water column has a critical responsibility for these results, because the concentrations of seston in the water column are almost 200 times lower than the SPM load (calculated by the overall median loads), while the mercury levels in SPM is only circa 6 times higher than the levels of mercury in seston.

In general, each mercury fraction showed a distinct seasonal and fortnightly pattern. Seasonally, it was observed that all mercury fractions had higher concentrations during winter regardless of the tidal amplitude. In addition to the seasonal influence, tidal forcing (through currents strength) and climate conditions were found to influence the mercury concentrations in both dissolved and particulate phases.

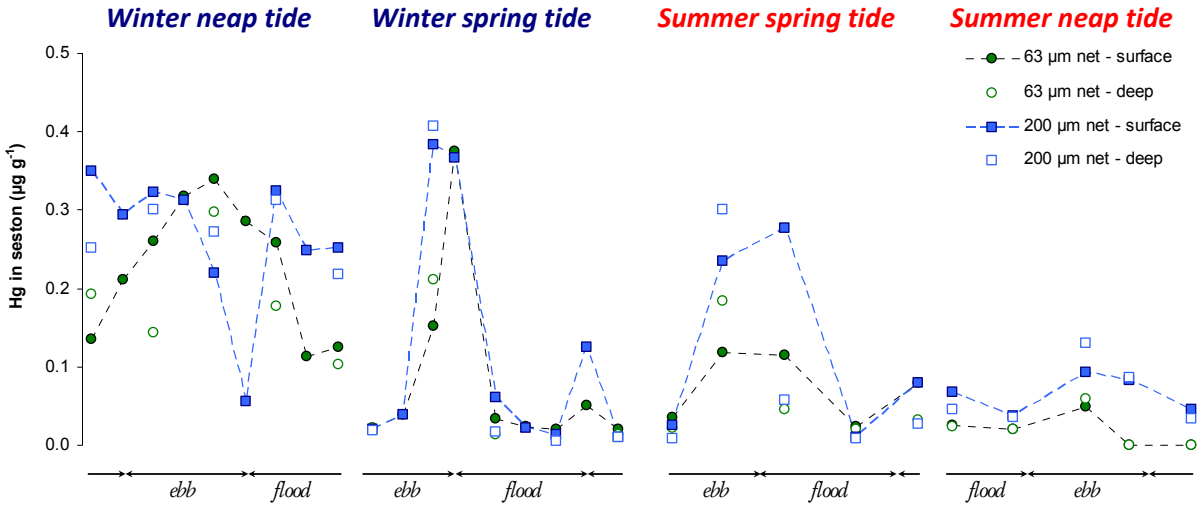


Figure 4.18 - Seasonal and tidal variation of total mercury (weight/weight) in seston collected with 63 µm and 200 µm nets during tidal cycles.

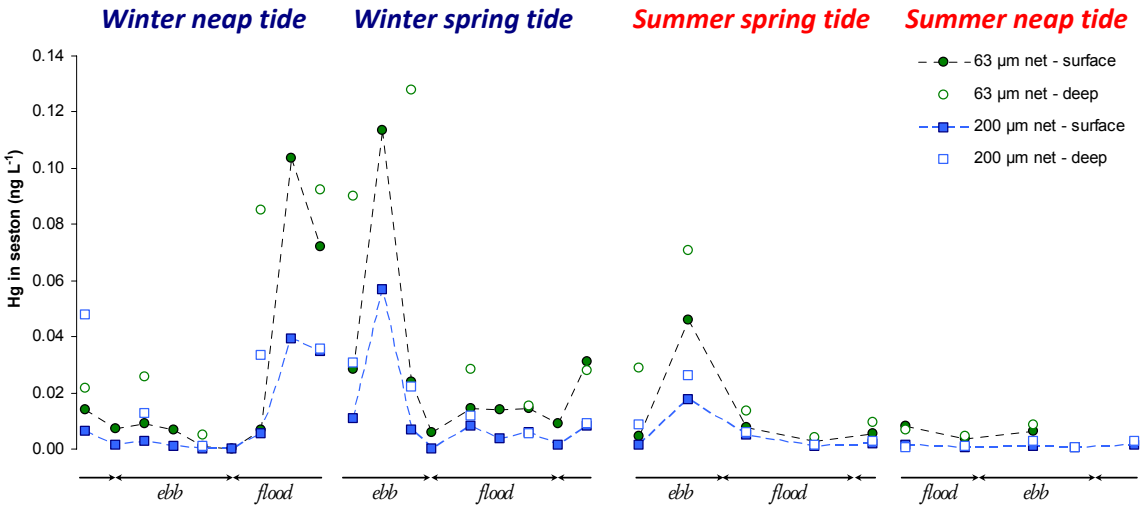


Figure 4.19 - Seasonal and tidal variation of total mercury (weight/volume) in seston collected with 63 µm and 200 µm nets during tidal cycles.

The concentrations of organic mercury in seston samples were only determined in the summer spring tidal cycle. The tidal variation is shown in Figure 4.20. The levels of organic mercury in seston ranged between 0.3 and 6.3 ng g^{-1}. Although not statistically significant, organic mercury concentrations were consistently higher in the seston collected with the 200 µm net, which can evidence biomagnification of the more toxic forms of mercury in the basis of the food chain. In terms of the fraction of mercury that is present as organic forms, higher values were also observed in the seston of higher dimensions, but never exceeding 5.1% of the total mercury. The organic fraction of mercury in estuarine waters is commonly very low (Wiener et al., 2003; Fitzgerald et al., 2007) and the observed fraction of organic species in seston collected in the Ria de Aveiro evidence bioaccumulation in the first steps of the food chain.

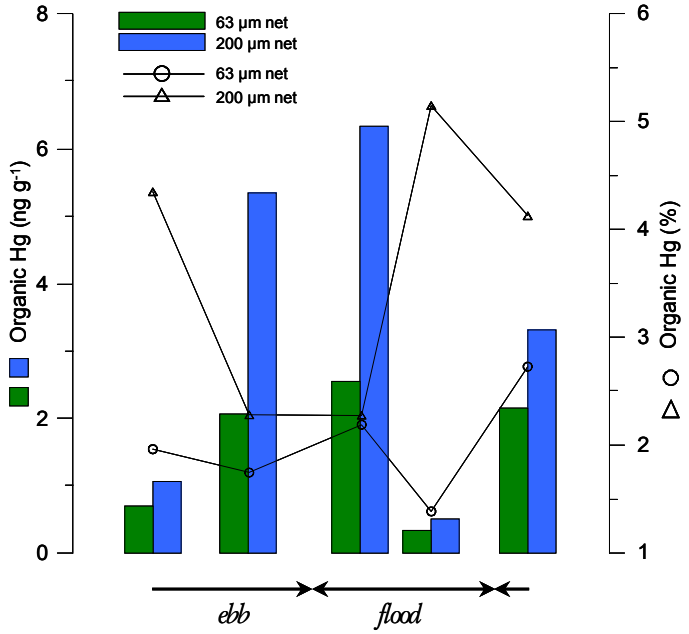


Figure 4.20 - Tidal variation of organic mercury concentrations (and percentage of mercury as organic species) in seston collected with 63 µm and 200 µm nets during the summer spring tidal cycle.

4.3.4 Mercury in the sediments

Bed sediments of the lagoon inlet showed very low fine fraction content (between 1.0 and 1.2%), organic content (between 0.2 and 0.6%) and mercury concentrations (ranging between 1.1 and 2.3 ng g^{-1}). Being mainly composed of coarse sediments (>63 µm) and depleted in organic matter, the bottom sediments of the channel have a weak capacity to trap mercury and the residence time of Hg in the outer boundary is short. Thus, it should be emphasised that, considering the sandy characteristics of the bottom sediments on the channel (Freitas et al., 2005), and contrary to the sediments in the contaminated basin, the sedimentary compartment in

the outer boundary of the lagoon does not act as a source or sink of mercury. In contrast with the finer solids of the more contaminated areas, the erosion and resuspension of these coarser sediments dilute the mercury content of the overall suspended solids.

4.3.5 Relationships between mercury distribution and master variables

The relationship between concentrations of trace metals and salinity are frequently used to describe the estuarine transit in terms of conservative or non-conservative behaviour.

Observing the distribution of total dissolved mercury concentrations as a function of salinity (Figure 4.21), it is not possible to distinguish a pattern in the range of salinities obtained. Even for the minima salinities (registered in winter), no implications for the dissolved mercury concentrations are observed.

For mercury in SPM (Figure 4.22), a decrease is observed in the concentrations, with increasing salinity ($r=-0.453$; $p<0.001$) and corroborates the presence of higher levels of mercury upstream and the transport of mercury down estuary to the Ocean.

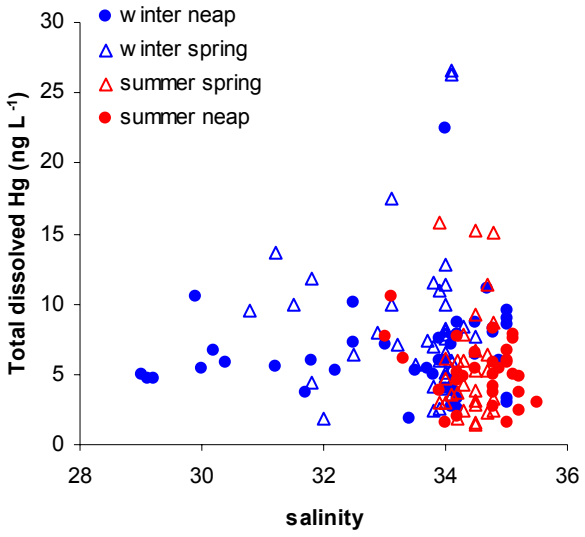


Figure 4.21 - Relationship between salinity and total dissolved mercury in the tidal cycles.

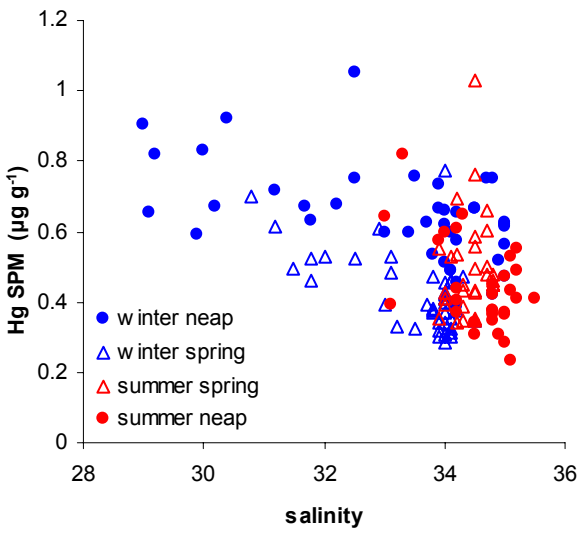


Figure 4.22 - Relationship between salinity and total particulate mercury in the tidal cycles.

This trend was also observed in the suspended particulate matter of Laranjo (Ramalhosa, 2002) and was considered to result from several factors, namely some dilution by the presence of mercury depleted particles arriving from the Ocean and from less contaminated areas of the lagoon, and also because of some mobilization of mercury from the particles to the dissolved phase with increasing salinity.

In the summer a decrease of mercury concentrations in SPM was observed with increasing concentrations of SPM (Figure 4.23), which is consistent with the observed decrease of POC (%) with increasing SPM (Figure 4.13) as a result of the presence of a diluting population of mercury and organic matter depleted particles. In fact, the distribution of mercury in SPM and POC (%) evidence a trend between the two variables (r=0.366; p=.004) during the summer (Figure 4.24). The organic enrichment of SPM is recognised as an important factor contributing to mercury concentrations in SPM (Choe et al., 2003; Laurier et al., 2003). The diverse significances between the two variables in the different tidal cycles suggest different nature and sources of organic matter.

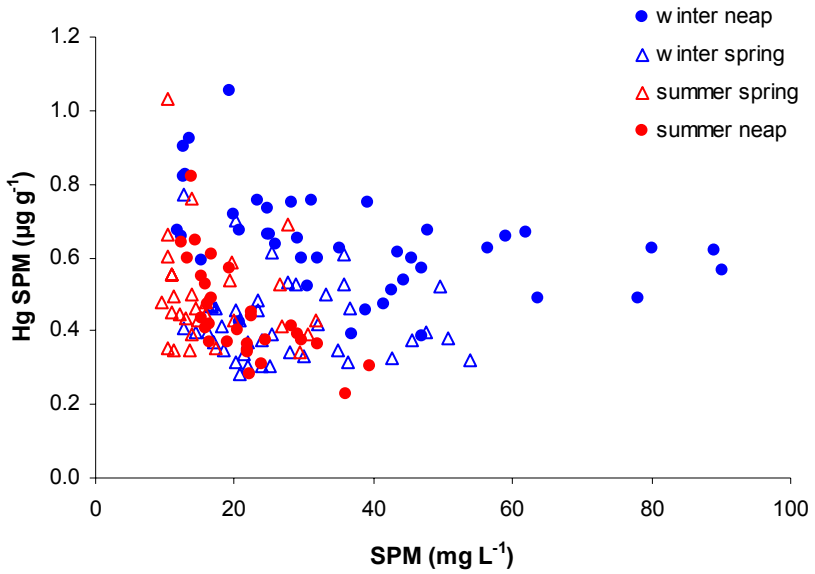


Figure 4.23 - Relationship between mercury in SPM and SPM concentrations in the tidal cycles.

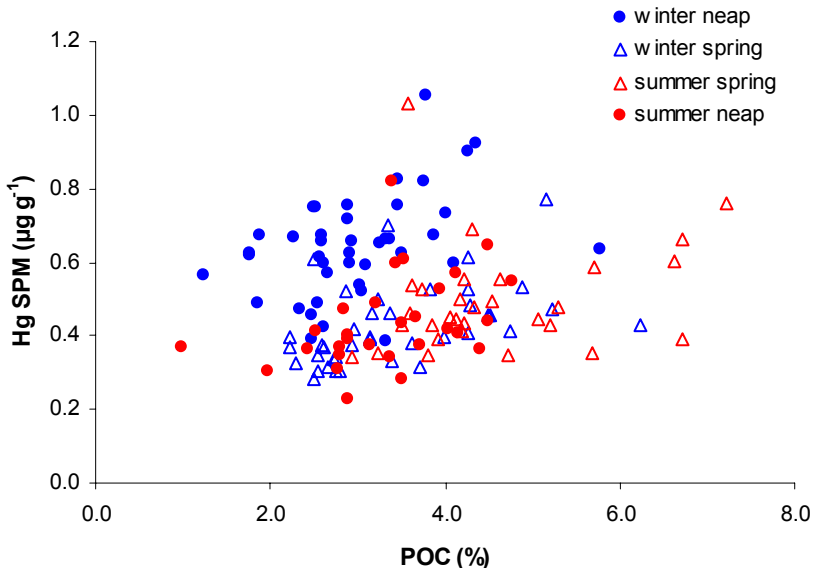


Figure 4.24 - Relationship between mercury in SPM and POC (%) in the tidal cycles.

4.3.6 Mercury partitioning in water column

The particle-water partitioning affects the reactivity, transport and fate of the mercury, turning the distribution between dissolved and particulate a key parameter in estuarine contaminant transport models (Turner and Millward, 1994; Lofts and Tipping, 2000; Johansson et al., 2001), especially considering the contrasting transport properties of dissolved and particulate forms of mercury. The analysis of the behaviour of the fraction of mercury that is associated with SPM (% particulate Hg) can be very useful to study the factors affecting the partitioning and was thereby chosen as the main representation in the present section (Johansson et al., 2001).

The partitioning of mercury between particulate and filter-passing fractions as a function of salinity is presented in Figure 4.25. Total particulate mercury in the inlet of Ria de Aveiro represented from 20 to 93% of total mercury (particulate + dissolved). In the salinity range observed, no evidence was found that salinity is the main factor controlling the partitioning. Nevertheless, the higher contributions of the dissolved mercury to the total amount in the water column were observed for high salinities, being the few occurrences of percentages of particulate mercury below 50% mostly observed for the more marine conditions observed during spring tides.

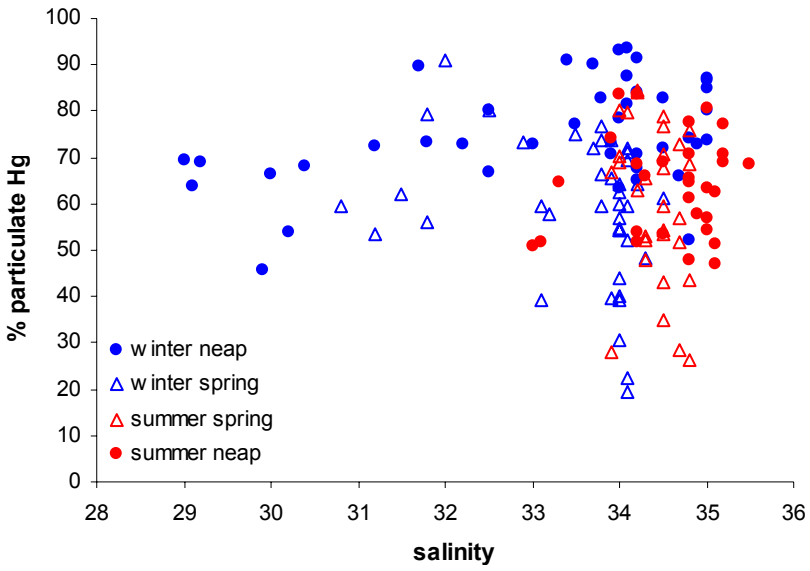


Figure 4.25 - Relationship between percentage of mercury in particulate phase and salinity in the tidal cycles.

This addition to the dissolved phase can be explained by the formation of chloro-complexes and by the competition of major ions by adsorption sites. Most of all, it can be observed that mercury is preferentially transported in the particulate fraction throughout the year.

An interesting relationship was found in the metal partitioning relative to the SPM concentration (Figure 4.26), with the mercury associated to the particulate fraction increasing with SPM concentrations. The contribution of particulate Hg to the total mercury is only below 50% when the SPM concentration is low (<30 mg L^{-1}) and increases non-linearly with increasing SPM concentrations. For the higher SPM concentrations, the mercury exists predominantly (80 - 90%) in particulate fraction. Even though the SPM concentration did not always explain the particulate Hg concentration (Figure 4.23), the relative abundance of particulate Hg appeared to be controlled by the SPM concentration to a significant extent, a behaviour also observed by Choe et al. (2003) in the San Francisco Bay estuary.

The resuspension observed during the winter neap tidal cycle and the consequent increased turbidity seemed to result in a higher association of mercury with the particulate phases, when compared with the winter spring tide conditions.

Although a mercury enrichment of suspended particles had been observed for lower concentrations of SPM, no evidence of particle concentration effect was observed.

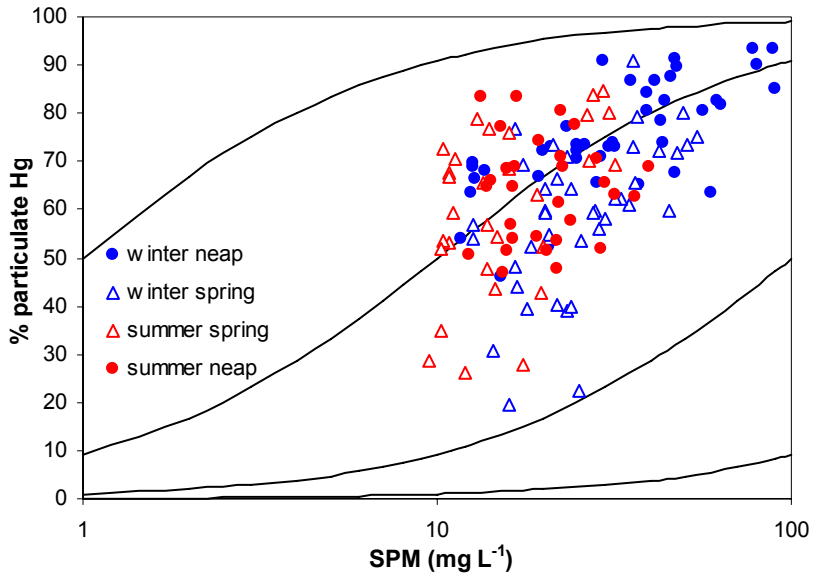


Figure 4.26 - Relationship between percentage of mercury in particulate phase and SPM concentration in the tidal cycles (the curves represent lines of constant K_D for which the $\log_{10} K_D$ values are indicated beside the line).

It is recognized the importance of organic content of SPM as a factor determining the dissolved-particulate distribution (Coquery et al., 1997; Lawson et al., 2001). The influence of organic matter in the partitioning of mercury can be observed in Figure 4.27, where it is possible to observe that the higher the fraction of organic matter associated with particles (relative to dissolved), the higher the affinity of mercury to particulate fraction.

The influence of POC (%) (Figure 4.28) in metal partitioning (Coquery et al. 1997; Lawson et al., 2001) showed some distinction between campaigns of different tidal range.

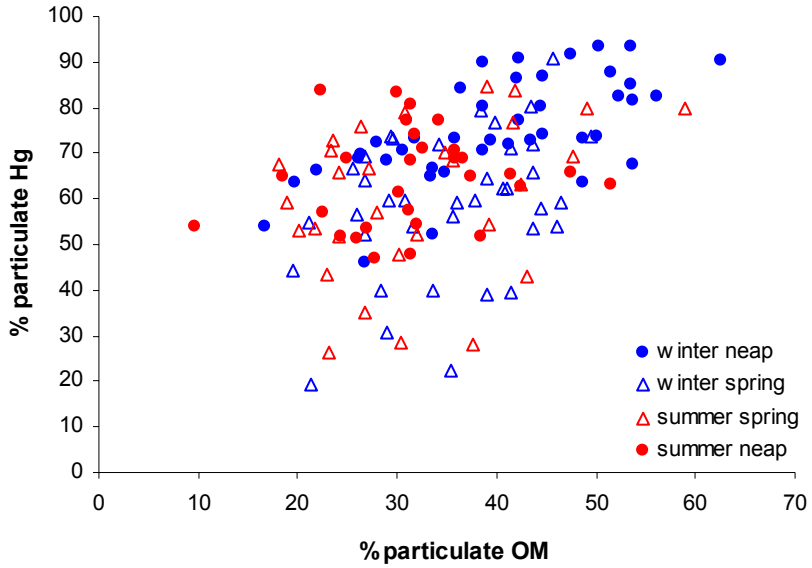


Figure 4.27 - Relationship between percentage of mercury in particulate phase and percentage of OM in particulate phase in the tidal cycles.

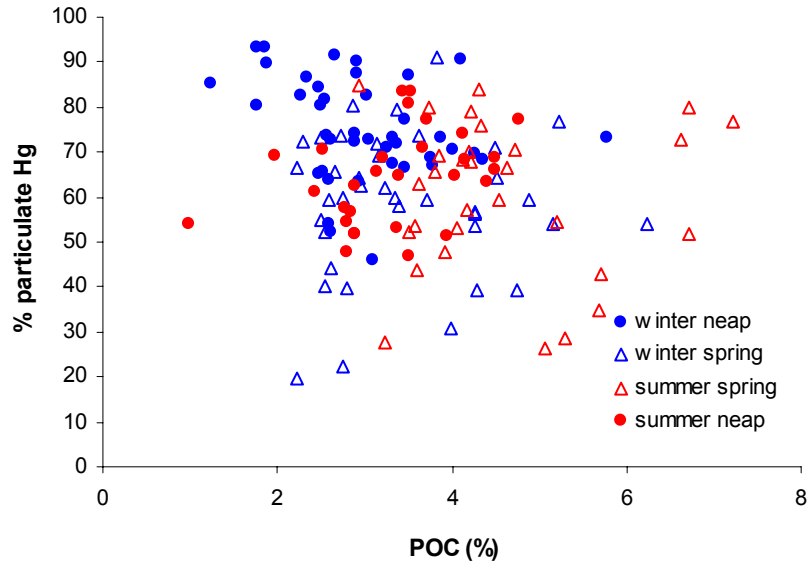


Figure 4.28 - Relationship between percentage of mercury in particulate phase and POC content (%) in the tidal cycles.

In fact, for a similar range of POC, a different partitioning is observed, implying that the nature of POC can be regulating the distribution. This indicates that fluvial organic matter, more usual during neap tides, can be in opposition to more marine like organic matter entering the lagoon during spring tides.

In organic-rich estuaries, an increase of the distribution coefficient with increasing salinity is often observed. Considering the good mixing of the end-members, the salting out of mercury has probably already been done in the upper reaches. No apparent salting out was evident for cadmium, lead, copper and zinc in the Ria de Aveiro in a salinity range between 0.2 and 35, probably due to the limited association of metals with the DOC (Monterroso, 2005).

The log K_D for the whole tidal cycles averaged 4.93 ± 0.27 (mean ± standard deviation; n=143). These are in agreement with the normal range for other European estuarine environments and coastal waters, being only slightly lower than some results reported in the literature (Coquery et al., 1997; Cossa et al., 2001; Leermakers et al., 2001; Laurier et al., 2003). This is true, especially for the winter sprig tide, where the average K_D (4.76 ± 0.26; mean ± standard deviation; n=41) was significantly lower than the averages of the other campaigns ($p<0.001$) probably as a result of a higher marine influence.

4.4 Estuarine mixing of mercury phases

Levels of mercury reported in works performed in the more contaminated zone of the lagoon are much higher than those observed in the present work (performed in the interface with the Ocean), where 96% of the determinations in the dissolved fraction were <15 ng L^{-1} and 88% <10 ng L^{-1} (demonstrating that higher values were not frequent). The salinity ranges during the tidal cycles and the behaviour of other parameters of the dissolved and particulate phases evidence a good mixing of the two end members (upper estuary and marine zone) in the channel.

The concentrations of total dissolved and particulate mercury levels during estuarine mixing in the Ria the Aveiro are shown in Figure 4.29 and Figure 4.30, respectively (error bars represent standard deviations).

For levels in the upstream area, the results reported by Coelho et al. (2005) were considered. For the open Atlantic Ocean, literature data was used (Cossa et al., 1996; Mason et al., 1998). The concentrations considered representative of the marine coastal zone are those obtained in the present study and detailed in Chapter 5. In Coelho et al. (2005), a quick drop of both dissolved and particulate concentrations was observed and even at 11 km from the estuary mouth (point of salinity ≈ 30 in Figure 4.29 and Figure 4.30) the levels already decreased by an order of magnitude.

This significant decrease of mercury concentrations in waters leaving Laranjo Basin is expected to have an important influence in the amounts of mercury transported by ebb tide to the main channel of the lagoon and ultimately to the Atlantic Ocean through the outlet channel.

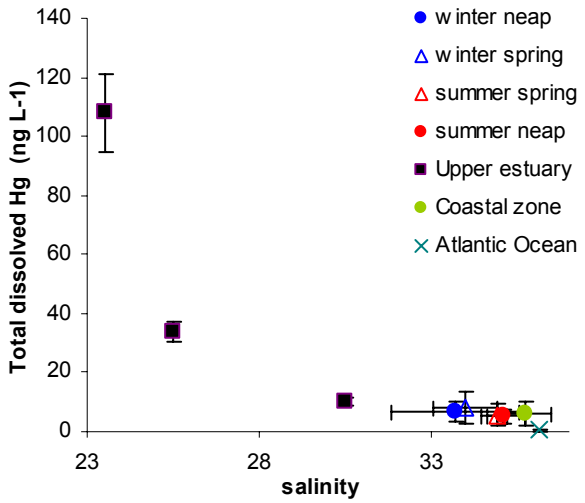


Figure 4.29 - Total dissolved mercury concentrations during mixing in the Ria de Aveiro.

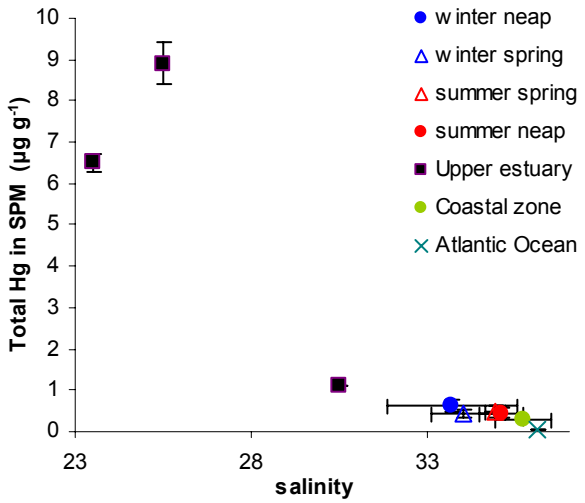


Figure 4.30 - Particulate mercury concentrations during mixing in the Ria de Aveiro.

4.5 Concluding remarks

The low salinity range observed during the surveys limited the observation of clearer relationships and the discernment between conservative and non-conservative behaviours. Furthermore, the examination of the importance of specific factors in the control of particle-water interactions was for this reason also restricted to some extent. An additional survey with a more representative transect between the two end-members is therefore required for clarification of some processes and relationships occurring in the lagoon.

A considerable variability was observed for mercury concentrations in seston and SPM during the tidal cycles and between tidal conditions. The assessment of such variability is important for the estimation of the amount of mercury transported from the estuary into the coastal sea. Several factors like tidal currents strength (related to tidal range), wind and river discharge were found to affect both the levels of mercury found in the water column phases and also the distribution between them. This influence was essentially related with processes affecting the resuspension of bed sediments, which acted as a source of mercury to the water column, when the resuspension occurred in the contaminated area, and also as a supply of diluting particles, decreasing thereby the mercury load of suspended particles.

Addition to the dissolved fraction was found to be enhanced by salt intrusion that moves upstream in spring tides, with an increase in ionic strength and competing ions (ion exchange and chloro-complexes formation). Different nature of POC and DOC during the year was probably also contributing to differences in partition.

5. Impact of the Ria input on the mercury distribution in the Atlantic coastal area

5.1 Introduction

The use of mercury (Hg) for industrial applications has contaminated many aquatic systems. The chlor-alkali industry has been one of the principal sources of mercury contamination, which was reduced in the latest two decades because many of the plants have switched from the Hg cell process to other technologies (Pacyna et al., 2001). Even after industrial discharges are reduced, large quantities of mercury remain buried in estuarine and coastal sediments which could be potentially released to coastal waters over the years. The biogeochemistry of mercury has received considerable attention due to the toxicity of methylmercury compounds (MeHg), the accumulation of mercury in biota, and its biomagnification in aquatic food chains. With respect to human exposure, it is generally accepted that the principal pathway is the consumption of contaminated fish, implying that special attention should be given to the concentrations in coastal zones, where fishing activities are most intense. Due to the strong binding of mercury to particles with organic coatings, about 98% of the mercury present in aquatic systems can be immobilised in the sediments (Kim et al., 2004). This association of mercury with particles retards its migration through interstitial waters to overlying water, allowing the sediment to act as a significant sink for mercury. Nevertheless, mercury associated with sediment can be remobilized by sulphide oxidation or complexation with organic ligands. In addition, through disturbances of the dynamic equilibrium between the sediment surface and the tidal currents, sediments and associated contaminants can be mixed in the water column and also be dispersed within the aquatic systems (Bloom et al., 2004; Kim et al., 2004; Pereira et al., 1998b). Remobilization can also be enhanced by other resuspension inducing phenomena, such as storms, dredging, and bioturbation (Rajar et al., 2004). The major role in the dispersion of contaminants is played by tides, recognized as the main forcing process in transport of suspended sediments (Lopes et al., 2001; Dias et al., 2003). Besides the tidal regime, the impact on shelf seas of mercury in estuarine sediments is governed by complex relationships between such factors as fluvial flow, terrestrial sediment load, local hydrodynamic conditions and the degree of contamination (Ridgway and Shimmield, 2002). The fate of Hg and MeHg in these sediments is a matter of concern, due to the potential for the transfer and dispersion of contaminants to adjacent coastal areas (Chiffoleau et al., 1999; Horvat et al., 1999; Stoichev et al., 2004).

In shallow waters, such as the case of the lagoon of Aveiro, the mercury cycle is dominated by direct coupling between the sediments and the food chain because they provide a large ratio of

sediment surface-to-water volume, in addition to frequent wind-driven and anthropogenic resuspension events (Bloom et al., 2004). Moreover, such systems are adjacent to highly productive intertidal wetlands, where significant microbial methylation takes place.

The main objective of the work presented in this chapter is to assess the importance (and seasonal variation) of the lagoon waters as carriers of mercury to the nearby coastal area. Therefore, the seasonal variability of mercury distribution in biota, surface sediments, and surface and deep waters of the coastal area were investigated.

5.2 Sampling design

Sampling of water and sediments was carried out on board NRP *Andrómeda*. Water samples were collected at eight sampling stations (Figure 5.1) located in Atlantic Ocean near the outlet of the coastal lagoon. One sampling station (Ref) was located at 11 kms from the lagoon mouth and was regarded as the reference point while station W8 was located in the channel separating the lagoon from the sea. Sampling was carried out during eight campaigns (September 2000, January and June 2001, March, July and December 2002, April and July 2003). Water samples were collected near the surface and at 15-20m depth. The exception was for station W8, where ship positioning and navigation restrictions prevented the collection of deep water.

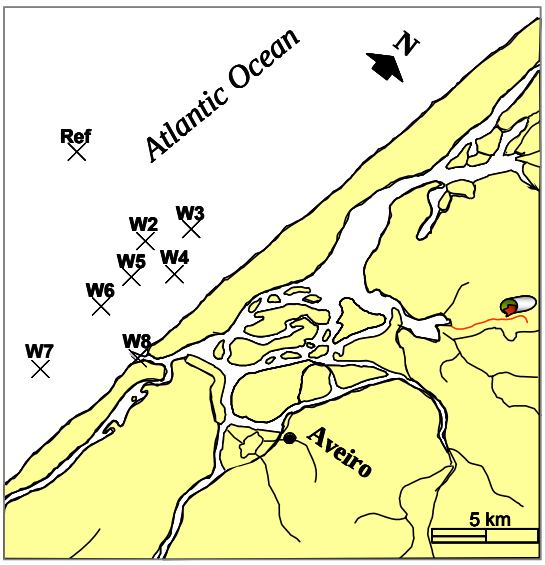


Figure 5.1 - Location of the water sampling stations (W2-W8 and Ref).

Sampling of surface sediments was carried out during four campaigns (September 2000, June 2001, March and December 2002) at twelve sampling stations (Figure 5.2). The locations of the sampling stations were intended to cover different distances from the outlet of the lagoon and

different distances from the coast, the latter having consequences in the depth of the sampling stations.

In February 2001, August 2001 and May 2003 biota were also collected in the vicinity of S. Jacinto and Torreira (Figure 5.2, site A and B, respectively). Species sampled were the lesser weever (*Echiichthys vipera*), the tub gurnard (*Trigla lucerna*), the wedge sole (*Dicologlossa cuneata*), the clam (*Spisula solida*) and the banded wedge shell (*Donax vittatus*).

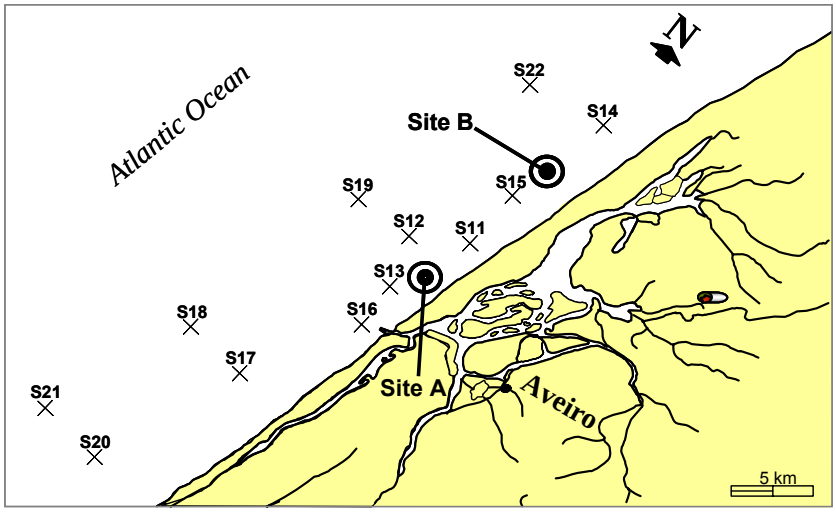


Figure 5.2 - Location of the sediment sampling stations (S12-S22) and biota sampling stations (Site A and B).

5.3 Results

5.3.1 Physico-chemical characteristics of the water column

The maximum temperature of the water (18.7 °C) was observed in June 2001 in the sample collected at the estuary mouth during the ebb tide. Surface water temperatures were statistically different (Wilcoxon matched pairs test; $p<0.05$) from deep water temperatures in all surveys, except in December 2002. The largest amplitudes between temperature of surface and deep waters were observed in summer surveys (June 2001 and July 2002 and 2003). Winter (January 2001 and December 2002) was the only season where the mean temperatures of deep waters were higher than those of surface waters.

Mean water pH was very similar in all sampling stations and between surveys with a small range of overall variation (7.9 - 8.3). The values observed in station W8 were nearly identical to the ones observed in the coastal zone, with pH in this station not statistically different from mean value for respective survey (Student's t-test; p-level of 0.05).

The salinities observed were similar at all sampling stations (Table 5.1), leading to a very narrow salinity range for each survey. Mean salinities were lower in winter surveys (January 2001

and December 2002), where values near 33 were observed contrasting with values higher than 35 in the remaining surveys. The lower salinities were observed in W8 during the ebb. Deep water salinities were always higher than salinities of surface waters.

Table 5.1 - Water salinity in the surveys (maximum, minimum and mean values for stations W2 to W7 and Ref) for surface and deep waters (at estuary mouth, station W8, only surface samples were collected, one during flood and another during ebb).

		Sep/2000	Jan/2001	Jun/2001	Mar/2002	Jul/2002	Dec/2002	Apr/2003	Jul/2003
Surface	Salinity	35.6	33.1	35.5	35.1	35.6	33.3	34.5	35.2
	max	35.7	33.7	35.5	35.3	35.8	33.8	34.9	35.4
	min	35.6	32.7	35.4	34.7	35.6	32.7	34.3	33.8
Deep	Salinity	35.8	35.1	35.7	35.8	35.7	33.8	34.9	35.7
	max	35.9	35.6	35.8	35.9	35.7	34.1	35.3	35.7
	min	35.7	33.8	35.4	35.8	35.7	33.5	34.7	35.7
W8 - Flood		--	--	--	35.0	35.8	33.5	34.0	35.6
W8 - Ebb		--	--	34.4	34.4	25.7	28.5	32.6	36.2

The suspended particulate material (SPM) concentrations were in the range 5-20 mg L^{-1}. The range of SPM levels in each campaign varied between surveys with a range of 2.4 mg L^{-1} in September 2000 and 11.1 mg L^{-1} in July 2002 (Figure 5.3).

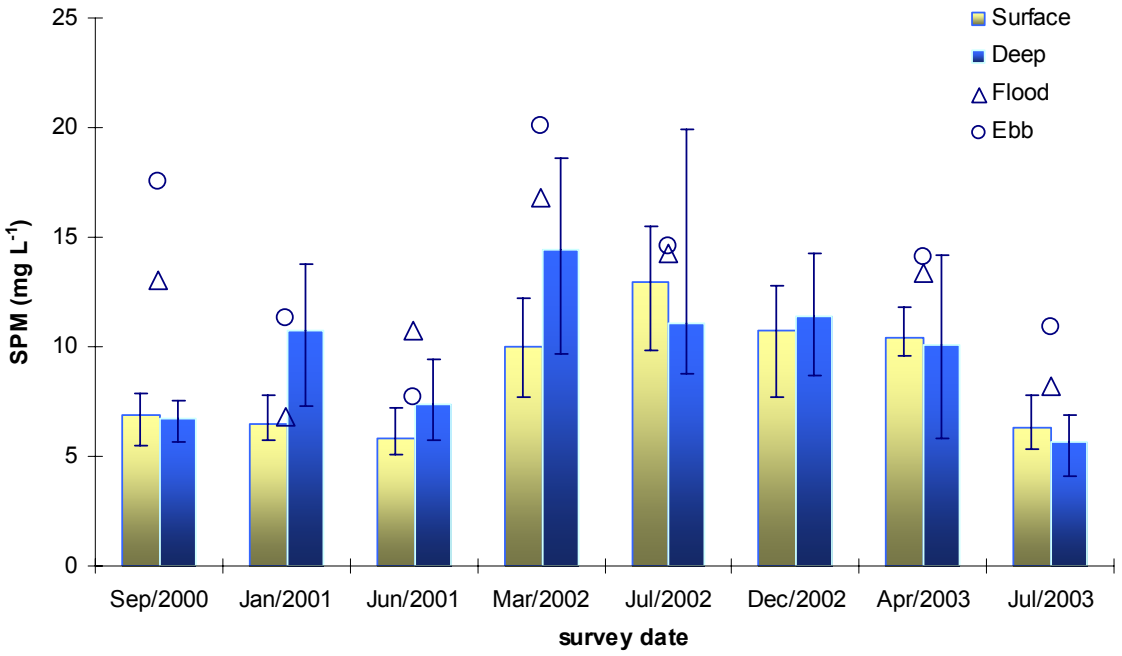


Figure 5.3 - Mean water SPM levels for each of the eight surveys (error bars indicate maximum and minimum values) for surface and deep waters (at estuary mouth - station W8 - only surface samples were collected, one during flood and another during ebb).

Concentrations observed at station W8 were always higher than the mean value of the remaining stations for the respective survey ($p<0.01$). The SPM concentrations observed at station W8 during the ebb were always higher (except in June 2001) than the value observed in the flood and in the coastal stations of the respective survey demonstrating the fluvial contribution of SPM to the coastal zone. Differences between the SPM concentration between surface and deep waters were observed in three campaigns between January 2001 and March 2002 (Wilcoxon matched pairs test; $p<0.05$).

5.3.2 Mercury in the water column (dissolved and particulate forms)

As shown in Table 5.2, reactive dissolved mercury concentrations observed during all the surveys were low and ranged from below detection limit to 9.8 ng L^{-1} with more than 80% of determinations below 3 ng L^{-1}.

Table 5.2 - Reactive dissolved mercury concentrations in the surveys (details of the legend as in Table 5.1).

		Sep 2000	Jan 2001	Jun 2001	Mar 2002	Jul 2002	Dec 2002	Apr 2003	Jul 2003
Surface	Reactive Hg (ng L^{-1})	1.5	2.1	4.5	2.7	2.0	1.2	4.1	3.1
	max	1.9	3.0	6.5	6.6	3.4	1.7	9.8	5.1
	min	1.1	1.0	1.1	1.2	< 1	< 1	1.2	1.2
Deep	Reactive Hg (ng L^{-1})	1.9	1.7	1.7	1.7	4.2	1.0	2.0	2.4
	max	2.2	2.5	2.5	2.6	9.1	1.1	3.3	3.4
	min	1.5	1.0	1.0	1.0	1.7	0.8	1.0	1.4
W8 - Flood		< 1	2.3	1.4	< 1	1.0	< 1	1.0	1.0
W8 - Ebb		2.5	2.1	1.8	1.2	3.2	0.9	--	1.4

Although the maximum values showed some variation over time, the mean concentrations were more constant. The concentrations observed at station W8 were lower for most of the time than the mean level of the respective survey. The total dissolved mercury concentrations at station W8 were similar to the mean values for each survey (Figure 5.4). Total dissolved mercury was not quantified in September 2000 and contamination during storage prevented the quantification of levels in April 2003. The total dissolved mercury concentrations ranged from 2 ng L^{-1} to 25 ng L^{-1} with more than 90% of results below 15 ng L^{-1} (and almost 80% of results below 10 ng L^{-1}). The overall average was 7.3 ± 1.1 ngL^{-1} (95% confidence; n=95).

Total mercury concentrations in SPM (Figure 5.5) ranged from 0.04 µg g^{-1} to 0.77 µg g^{-1} with 84% of the values between 0.1 and 0.4 µg g^{-1}. The overall average was 0.28 ± 0.02 µg g^{-1} (95% confidence; n=119). In most of the surveys (except for summer 2002) SPM mercury concentrations at station W8 were higher during ebb than the mean level for the remaining sampling stations.

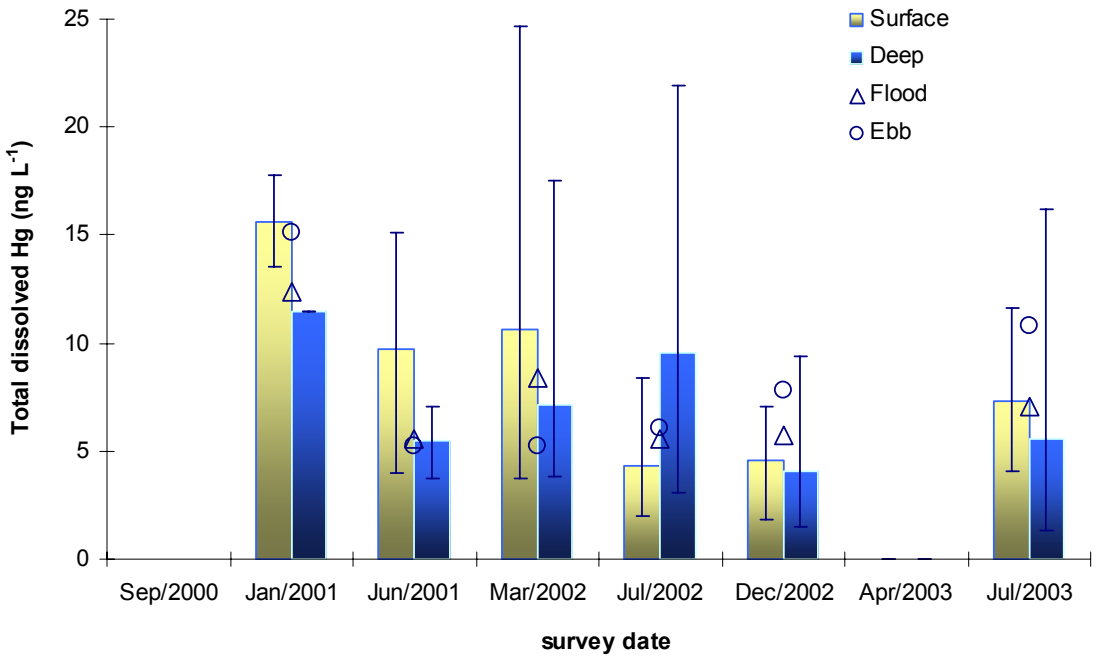


Figure 5.4 - Mean total dissolved Hg concentrations for each of the eight surveys (details of the legend in Figure 5.3).

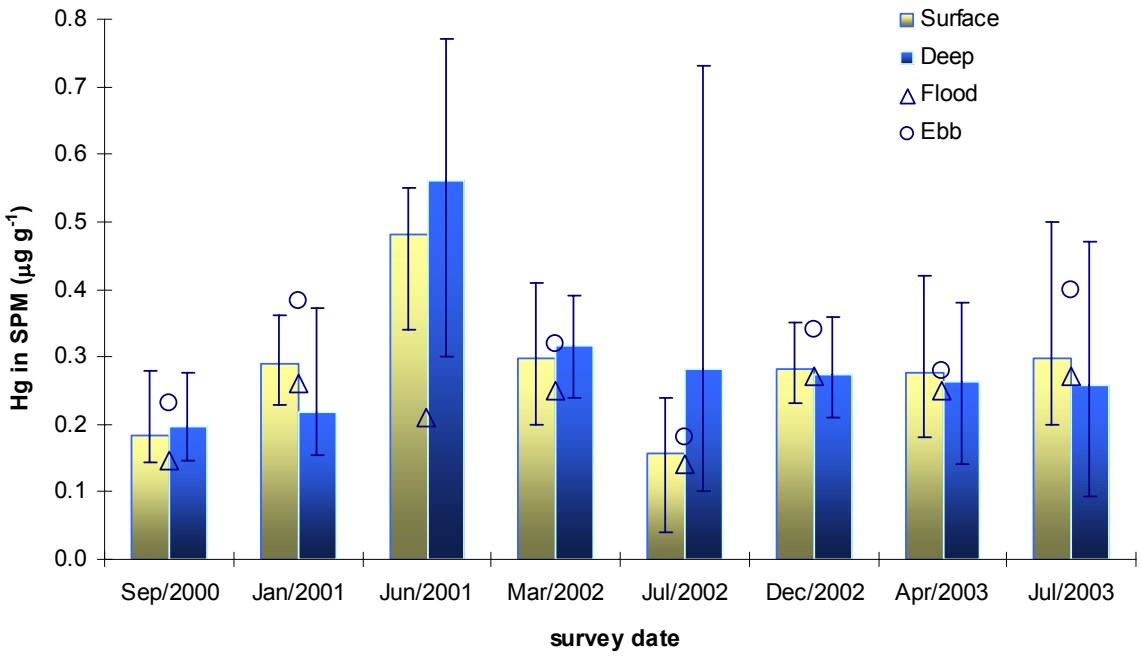


Figure 5.5 - Mean SPM Hg concentrations for each of the eight surveys (details of the legend as in Figure 5.3).

5.3.3 Mercury in the sediments

Total mercury concentrations in the sediments indicate that in September 2000 values ranged from 1.7 to 7.3 ng g^{-1}, as shown in Figure 5.6, and slightly lower concentrations were found in June 2001 ranging from 1.0 to 4.6 ng g^{-1}. In March 2002 and December 2002 values were always higher than 1.0 ng g^{-1} and lower than 8.6 ng g^{-1} and 6.6 ng g^{-1}, respectively. The overall average concentration in all surveys was 3.72 ± 0.63 ng g^{-1} (95% confidence; n=44). The carbon content of sediments varied from 0.033% to 0.49% with overall average of 0.31 ± 0.03 % (95% confidence; n=44). The fine fraction (< 63 µm) ranged between 0.9% and 7.7% with an overall average of 3.4 ± 0.5 % (95% confidence; n=44).

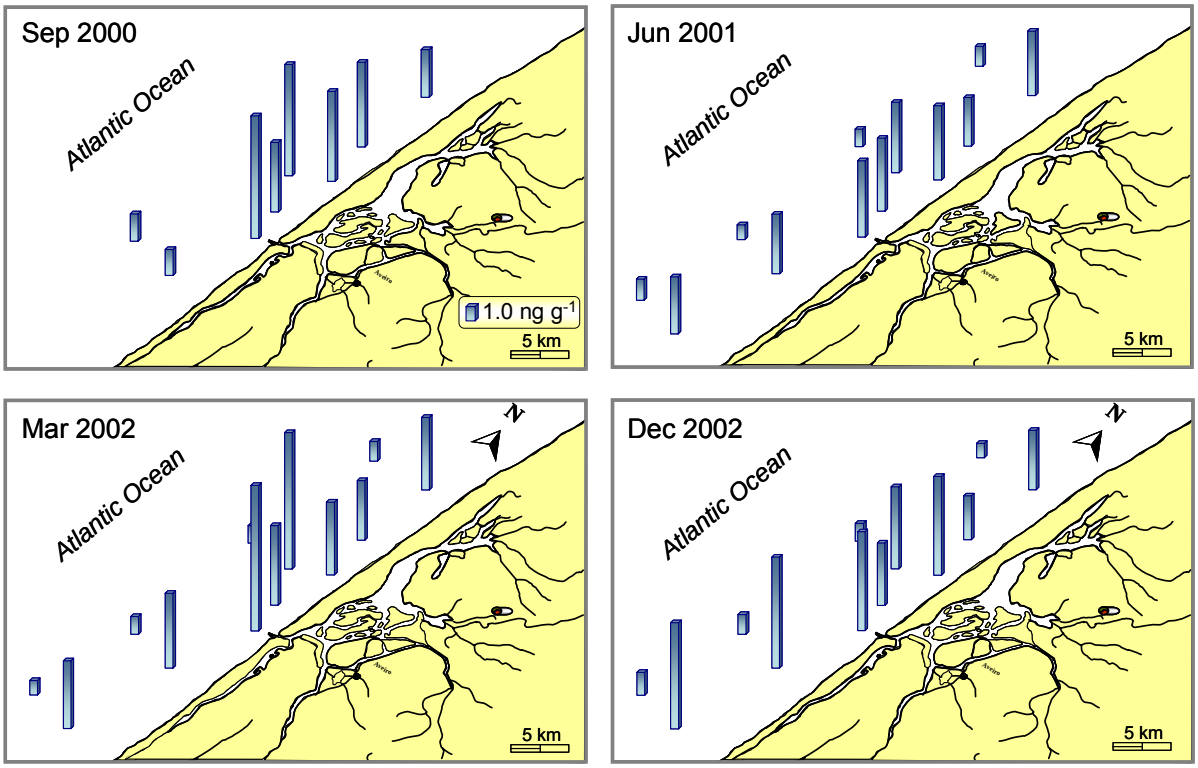


Figure 5.6 - Mercury concentrations in surface sediments in September 2000, June 2001, March 2002 and December 2002.

5.3.4 Mercury in biota

Concentrations of total mercury in organisms (dry weight) collected in S. Jacinto and Torreira (respectively, site A and B in Figure 5.2) are presented in Figure 5.7. Due to the longer distance to the mouth of the lagoon, location B should be less affected by mercury arriving from the lagoon.

In S. Jacinto, the highest mercury concentrations were found in wedge sole tissues with values that ranged in muscle from 0.42 (dw) to 0.82 µg g^{-1} and in liver from 0.25 to 0.67 µg g^{-1}. Tub

gurnard had the lowest mercury levels with concentrations in muscle ranging from 0.13 to 0.26 µg g^{-1} and in liver from 0.096 to 0.19 µg g^{-1}. For banded wedge shell concentrations ranged from 0.056 to 0.13 µg g^{-1}.

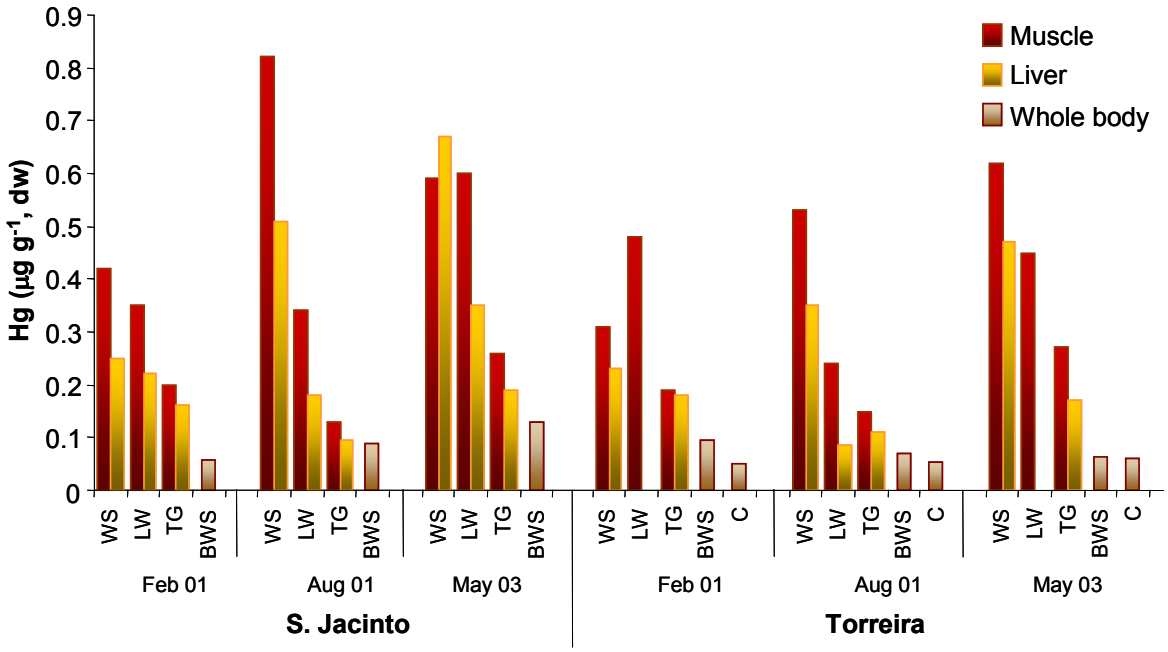


Figure 5.7 - Total mercury concentrations in organisms collected at S. Jacinto and Torreira in February 2001, August 2001 and May 2003: WS (wedge sole), LW (lesser weever), TG (tub gurnard), BWS (banded wedge shell) and C (clam).

Total mercury concentrations in organisms collected from Torreira showed a similar trend to the one observed in S. Jacinto, with wedge sole and lesser weever displaying the higher concentrations. In these two fish species values ranged in muscle from 0.24 to 0.62 µg g^{-1} and in liver from 0.084 to 0.47 µg g^{-1}. Concentrations in the two bivalve species were nearly identical with mercury levels that ranged from 0.052 to 0.04 µg g^{-1} (overall average concentration of 0.065 ± 0.016 µg g^{-1} (95% confidence).

5.4 Discussion

5.4.1 Mercury partitioning in water column

The small range of the mean values of concentrations for the several surveys indicated no clear seasonal pattern in reactive dissolved levels of mercury. In a study on mercury about 200 km north from Aveiro lagoon, Beiras et al. (2002) found reactive dissolved concentrations of mercury one order of magnitude higher than those observed in the coast of Ria de Aveiro. In the case of total dissolved mercury, except for July 2002, surface mean levels were always higher than deep

mean concentrations (p=0.019; paired t-test). Even in the campaign when the highest maximum occurred (March 2002) 70% of the determinations were lower than 10 ng L^{-1}. When the second highest maximum occurred (July 2002) more than 85% of the determinations were lower than 10 ng L^{-1} demonstrating that these higher values are not frequent. Inside the lagoon, in the most contaminated area, concentrations of reactive and total dissolved mercury were found to be up to 84 ng L^{-1} and 275 ng L^{-1}, respectively (Coelho et al., 2005; Ramalhosa et al., 2006a). Concentrations found in the coastal zone are therefore low and one to two orders of magnitude lower than those inside the lagoon.

The fraction of reactive dissolved mercury in this study (in 90% of the results the reactive fraction of dissolved mercury was in the range from 10% to 60%) was higher than that observed in Brazilian coastal lagoons by Lacerda and Gonçalves (2001) who found that only 2% of dissolved mercury was present in reactive forms (due to a strong association of mercury with dissolved organic complexes).

Except for June 2001, mean mercury concentrations in SPM were relatively constant over the time, with no marked difference between surface and deep mean levels. According to Wilcoxon matched pairs test (p-level of 0.05) no differences in concentrations were observed between the surface and deep waters in all campaigns. Seasonal differences in the Hg concentrations of particulate matter were likely to occur (Kruskal-Wallis One Way Analysis of Variance on Ranks; $p<0.001$;) since the metal uptake in the biogenic fraction (which could be significant) can vary markedly with the season (Vasconcelos and Leal, 2001). Higher SPM mercury concentrations at the estuary mouth during ebb highlight the transport of mercury to the coastal zone.

Ramalhosa et al. (2001) studied the concentrations of mercury in the most contaminated area of the Ria, a basin (into which the chlor-alkali effluent was discharged) with several tons of mercury buried in the sediments (Pereira et al., 1998a). The mercury concentrations in SPM inside the basin (3.5 - 26.0 μg g^{-1}) are one to two orders of magnitude higher than those found in the present study of coastal waters (0.28 ± 0.02 μg g^{-1}). This high mercury load in suspended particles inside the lagoon is explained by the high total mercury concentrations of surface sediments in the mentioned contaminated area (up to 40 μg g^{-1} according to Ramalhosa et al. (2005). During periods of stronger currents and bottom resuspension mercury can be exported from this contaminated area to the rest of the lagoon (Pereira et al., 1998b) towards the estuary mouth.

Leermakers et al. (2001) observed higher total dissolved concentrations in winter than in summer and the reverse for particulate mercury concentrations. However, in this study, no link was observed between mercury concentrations and season. In spite of the maximum

concentrations of particulate mercury were observed in summer 2001 (June), in the other two summer surveys (July 2002 and July 2003) concentrations were very similar to the ones observed in the remaining campaigns.

Figure 5.8 shows the percentages of mercury in the particulate phase (calculated as the ratio between the volumetric total mercury concentration in the particulate phase and the sum of the volumetric concentrations in dissolved (reactive or total Hg) and particulate phases) against SPM, together with isopleths of log K_D.

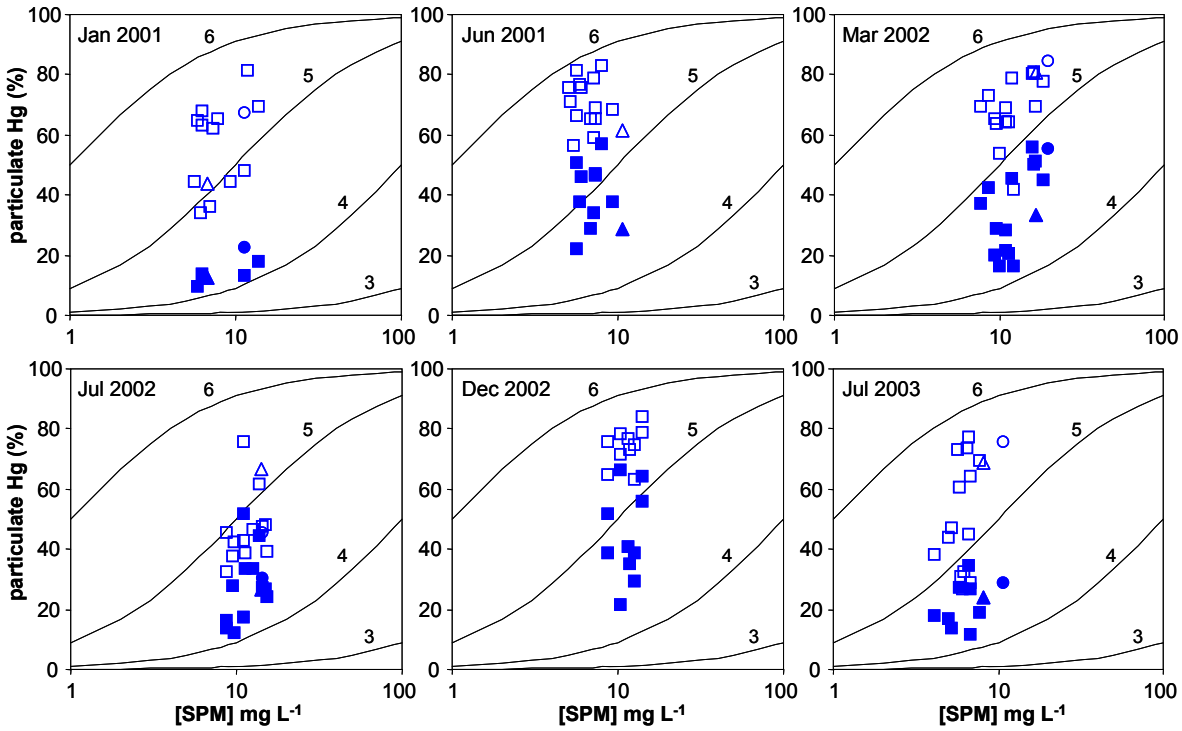


Figure 5.8 - Variation in percentage of particulate mercury with SPM concentration in the six surveys. Curves represent lines of constant K_D for which the log10 K_D values are indicated beside the line. Full symbols represent results calculated with total dissolved mercury and open symbols refer to partitioning calculated with the reactive fraction of the dissolved fraction (squares indicate coastal samples, circles and triangles indicate samples collected at estuary mouth during ebb and flood, respectively).

Considering the partitioning calculated by taking into account the total dissolved mercury, results show that, in general, mercury is mainly associated with the dissolved phase (45-90%), which can be determinant to the bioavailability of mercury to marine species. Also important to the impact to biota is the fact that reactive forms of dissolved mercury are sometimes an important fraction of dissolved mercury in these waters. The $\log_{10} K_D$ averaged 4.64 ± 0.07 (95% confidence; n=68), but is lower than values (5.0-5.8 and 5.4 ± 0.4) reported for other European coastal waters in studies reviewed by Coquery et al. (1997) but similar to values observed by Leermakers et al. (2001) in Belgian coastal waters. No difference was found between surface and

deep waters for values of log K_D (paired *t*-test; p= 0.760). Although some differences in K_D were observed between campaigns, no clear seasonal pattern could be identified. For example, minima values of K_D were observed in the winter survey in 2001 and maximum values of K_D were found in the winter survey in 2002. In spite of this variation between campaigns, no statistically significant seasonal effect such as the one observed by Leermakers et al. (2001) could be found. In contrast with this study, Leermakers et al. (2001) noted variation of both dissolved and particulate concentrations resulted in differences in the K_D values, with higher values occurring in the summer.

No significant relationship was found between distribution coefficient and particulate matter concentrations. Variations in the partition between the dissolved and particulate phases can therefore be assigned to variations in mercury load of particles and/or variations in dissolved levels. The results for partition coefficient at the estuary mouth (sampling station W8) are comparable with the ones observed in the coast, showing that the partition is not significantly changed in the coastal waters.

Mercury concentrations found in this study are compared in Table 5.3 with other coastal marine data from the literature. Horvat et al. (1999) reviewed the mercury concentrations in ocean and coastal waters and presented a range for dissolved mercury of 0.5 – 2.0 ng L^{-1} in near shore coastal areas and 0.2 – 0.4 ng L^{-1} for off shore waters.

Table 5.3 - Mercury concentrations in water and sediments in this study compared with coastal values from the literature.

Location	Hg reactive (ng L^{-1})	Hg Total (ng L^{-1})	SPM (µg g^{-1})	Sediments (ng g^{-1})	References
Portuguese Western Coast	1.0 – 4.5 [a]	4.1 – 15.7 [a]	0.18 – 0.56 [a]	1.0 - 8.6 [a]	This work
Belgian coastal zone	-	0.1 – 1.9	0.02 – 0.4	3.23 – 28 (sandy sediments)	Leermarkers et al., 2001
Sub-polar North Atlantic	-	0.48 ± 0.32	-	-	Mason et al., 1998
Equatorial and South Atlantic	0.34 ± 0.24	0.58 ± 0.34	-	-	Mason and Sullivan, 1999
Bay of Byscay	-	-	-	20 - 460 (average: 160)	Stoichev et al., 2004
Gulf and Gulf of Oman	-	-	-	< 1 – 16.7	de Mora et al., 2004
Atlantic coast of southern Spain	-	-	-	110 - 410	Usero et al., 2005

[a] Range of means

As for particulate mercury, the same authors report that in near shore coastal areas the levels ranged between 0.04 and 1.88 µg g^{-1}. The abundance of particulate mercury in coastal waters makes these regions prone to have higher mercury concentrations compared with the open sea (Cossa et al., 1996). In this study, dissolved mercury concentrations are higher than those observed in other sites but mercury in SPM are comparable with concentrations found, for example, in Belgian coastal zone.

5.4.2 Mercury in sediments

Significant differences between sampling stations (two-way ANOVA; $p<0.001$) were observed in all campaigns. Besides, sample stations closer to the coast showed significant higher levels than the others ($p<0.001$ in two campaigns and $p<0.03$ in the other two), and differences between samples from near coast and offshore are displayed in Figure 5.9.

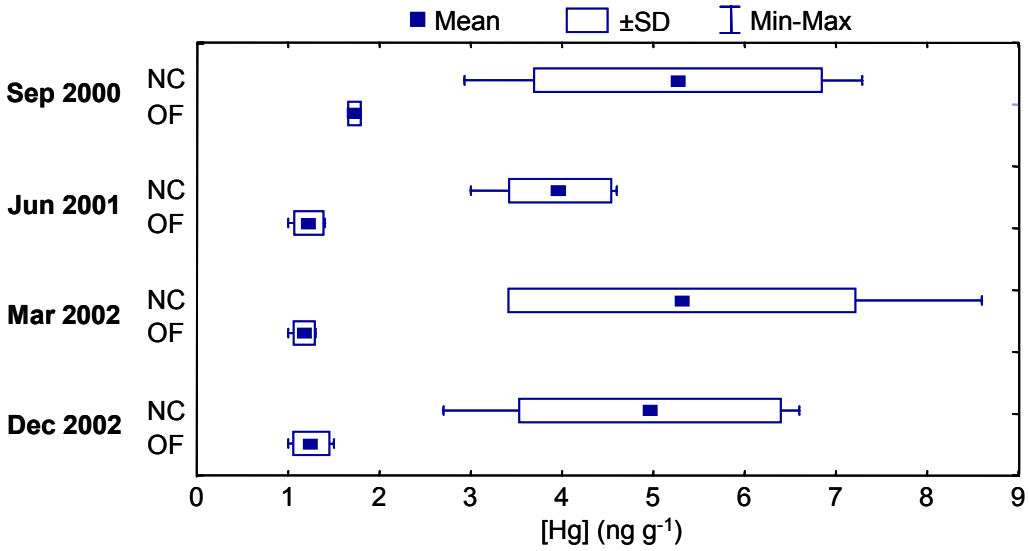


Figure 5.9 - Box and whisker plots of surface sediments mercury concentrations in the four campaigns and in two groups of sampling stations: near coast (NC) and offshore (OF).

In three of the four campaigns a statistically significant relationship was found between total mercury in sediments and the fine fraction content ($p<0.0002$-0.02), highlighting the importance of grain size in the mercury accumulation. However, no significant correlation was found between mercury content and carbon content in sediments (p-level of 0.05). About 200 km to the north, Beiras et al. (2002) found much higher levels of Hg in the coastal sediments, on the order of some hundred of nanograms per gram, and in some samples, reaching values of 1000 to 2000 nanograms per gram of sediment.

In spite of the well established role of sediments as a sink (and as a source) of contaminants in aquatic systems, the low mercury levels observed can be explained by the inefficiency of coastal sediments in trapping mercury coming from the lagoon, because of their low carbon levels and low fine fraction content.

Mercury concentrations found in coastal sediments in this study (ranging between 1.0 and 8.6 ng g^{-1}) are well below the provisional lower environmental assessment criteria (EAC) of 50 ng g^{-1}. EACs were established recently by OSPAR to express biological and/or ecotoxicological risk (Bignert et al., 2004). Values below the lower EAC threshold should not give rise to any adverse biological effects.

Levels in sediments obtained in this study are also very low when compared with those found in other sites, namely in the European Atlantic coast, as shown in Table 5.3. Thus, Hg concentrations in coastal marine sediments nearby Ria de Aveiro are generally quite low when compared with international standards.

5.4.3 Mercury in aquatic biota

In S. Jacinto (site B in Figure 5.2), differences (p=0.005) in total mercury concentrations between species were found, with wedge sole showing the highest levels. The banded wedge shell and the liver of wedge sole appear to display an increase of mercury concentrations with time. In spite of these considerations, and although the seasonally dynamic nature of mercury accumulation (Post et al., 1996; Greenfield et al., 2005) - important variables of mercury uptake such as temperature, body size and diet vary substantially with season - considering all species, no statistical difference was observed between sampling dates (two-way ANOVA; p=0.16 in Torreira and p=0.06 in S. Jacinto). For the three sampling dates, there is no evidence of important differences between mercury levels in both locations (two-way ANOVA; p-level of 0.05). Inside the lagoon the concentrations of Hg in muscle of sea bass (Abreu et al., 2000) ranged from 0.03 to 1.7 μg g^{-1} (fresh weight), with the highest values found in individuals captured in the most contaminated basin. Levels observed in the tissues of the bivalve species studied (0.065 ± 0.016 μg g^{-1} (dw); 95% confidence) are from 1 to 10 times lower than the values found by Beiras et al. (2002) in 1998 in mussels from the Galician coast, reflecting the low contamination of the sediments in the marine coastal area of Aveiro. The assessment of mercury concentrations in bivalve molluscs is especially important once these organisms are used as biomonitors because of their ability to accumulate trace pollutants in their tissues in response to the bioavailable chemical forms (Beiras et al., 2002; Morillo et al., 2005; Usero et al., 2005).

Levels observed in bivalves are lower than the range of mean value of mercury concentrations reported along the Portuguese coast [0.08 – 0.48 μg g^{-1} (dw)] and even in the rest of the respective OSPAR region (OSPAR, 2000). According to European Union regulations in this matter (No 466/2001 of 8 March 2001) the maximum level of mercury in foodstuff is 0.5 μg g^{-1} (fresh weight). Our results have shown total mercury concentrations, expressed in dry weight, ranging between 0.82 and 0.05 μg g^{-1} (average of 0.28 μg g^{-1} and standard deviation of 0.21 μg g^{-1}). To compare with the European Directive one must regard the moisture content which ranged in our samples between 70% and 80% with an average of 76.1% and a standard deviation of 5.1% (n=43). Accounting for these results, mercury levels on a fresh weight basis are well below the threshold limit of concentrations allowed in edible parts of marine species.

Considering the levels of Hg in water, the coastal organisms showed evidence of bioaccumulation assessed by the calculated bioaccumulation factors: for fish between 8000 and 18000 and for bivalves between 1000 and 2000. Assuming that fish contain levels resembling those found in muscle of lesser weever and wedge sole (0.6 μg g^{-1}, dw) and assuming also that almost all mercury in fish is found as methylmercury, a consumption of 200 g of these fish account for ⅓ of the recommended provisional tolerable weekly intake (PTWI) for a 60 kg person (WHO, 2007). On the contrary, considering the mercury levels in fish from the inner contaminated area of the lagoon (e.g. 1.2 μg g^{-1}, ww; Abreu et al., 2000), the ingestion of 80 g of a 15 cm fish would fulfil the PTWI.

In fish, only one occurrence of higher mercury concentrations in liver than muscle was observed (n=18). The ratio between liver and muscle mercury levels can reflect the exposure history of organisms, being lower than 1 in fish with long-term exposure, while in fish individuals with recent exposure, it should be higher than 1 (Beckvar et al., 1996). Based on results of several studies Goldstein et al. (1996) suggested that when concentrations in muscle are low (less than about 0.5 μg g^{-1}), levels in muscle are double those observed in liver, and when higher concentrations are reached (higher than 1 μg g^{-1}) in muscle, this ratio is inverted due to a redistribution from the muscle and concentrations in liver can be several times higher than those found in muscle. In the present work no differences (p=0.844) between S. Jacinto and Torreira were observed in liver to muscle ratios suggesting that stress exposure is similar in both sampling stations studied. The ratio ranged from 0.35 to 1.1 with overall mean of 0.7 ± 0.1. Abreu et al. (2000) found, in the most contaminated area inside the lagoon, levels in the liver of sea bass that were almost double those in muscle. In less contaminated areas, concentrations were similar in both tissues, with the ratio being on the order of 0.4 in a branch of the lagoon recognized as less

contaminated with anthropogenic mercury (a station close to the sea). The ratios observed in the present study for coastal fish thus suggest that fish studied are more exposed along the coast than in some sites inside the estuary. Mercury concentrations found in this study in fish and bivalves tissues were compared with other data from the literature, as shown in Table 5.4, leading to the conclusion that values obtained for this work were within the range found in other sites.

Table 5.4 - Mercury concentrations in biota in this study compared with coastal values from the literature.

	Biota	Hg Total (μg g^{-1})	
Portuguese Western Coast	Muscle tissue of fish	0.13 - 0.82 (dw)	This work
	Bivalves	0.052 - 0.13 (dw)	
Atlantic coast of southern Spain	Mollusc bivalve (*D. trunculus*)	Average: 0.12 (dw)	Usero et al., 2005
	Mollusc bivalve (*C. gallina*)	Average: 0.05 (dw)	
Bohai Sea, China	Molluscs	0.005 - 0.199 (ww)	Yawei et al., 2005
Gulf of Mexico	Edible tissue of fish	Average: 0.40 (ww)	Lewis et al., 2002
Arabian Sea, Pakistan	fish	0.09 - 0.16 [a] (ww)	Tariq et al., 1993
Gulf and Gulf of Oman	fish	Muscle: 0.50 – 2.35 (dw) Liver: 0.287 – 4.65 (dw)	de Mora et al., 2004
	bivalves	0.0087 – 0.315	
North East Coast of England	Muscle tissue of fish	0.028 – 0.141 [a] (ww)	Dixon and Jones, 1994

[a] Range of means

5.5 Conclusions

The high mercury concentrations found in many aquatic systems, as a result of past anthropogenic emissions, should make the assessment of both its transport and dispersion to nearby coastal zones a priority. The hydrological circulation in the Ria de Aveiro is essentially dominated by tidal forcing, which together with the high levels of mercury found inside some areas of the lagoon results in a daily mercury transport to the coastal zone during the exchange of water mass. However, there is a small impact on the nearby marine area on a scale of several kilometres. This can be explained by the extensive and complex web of islands, and meandering interconnected channels that makes water circulation inside the lagoon difficult (Lopes et al., 2001), allowing the spreading and trapping of contaminants before they are discharged into the coastal waters through the single narrow connection to the sea.

Mercury related determinations shows no seasonal pattern in all the compartments studied. Although organisms exhibited some bioaccumulation of mercury, levels are below regulatory guidelines with no evidence of mercury pollution posing a threat to human health in the marine coastal zone. Levels rise above background reference concentrations only in the water column, particularly in SPM due to the high affinity of mercury for particulate matter. The long range transport of these small dimension particles together with the low efficiency of sandy sediments to trap mercury will play an important role in the dispersion of mercury over a broaden area and consequently leading to lower values in the zone near the estuary mouth.

6. Mercury exchanges between the Ria de Aveiro and the Atlantic Ocean

6.1 Introduction

Estimating fluxes of mercury to coastal zones is essential to evaluate the fate of mercury remobilized, the recovery of contaminated estuaries and the impact in the coastal marine areas and in the regional mercury cycles. Estimates of exchanges to coastal waters are also very important to implement the regulations from the international conventions which intend to reduce the terrigenous contaminant inputs to the sea.

Generally, trace metal budgets are based on assessment of annual fluxes taking in account accurate measurements of water discharge and SPM (suspended particulate matter) but fewer data of chemical concentrations, typically mensal samplings (Hissler and Probst, 2006; Schäfer et al., 2006, Leitch et al., 2007). Semidiurnal and fortnightly (spring vs. neap tides) behaviour is generally neglected.

In order to understand the fate of mercury in the lagoon with a single connection to the Atlantic Ocean, the present study aimed at assessing: (1) how much of the mercury within the system reaches the Ocean; (2) which is the most important compartment for mercury transport (dissolved, particulate or seston, which correspond to particulate matter such as plankton, organic detritus and inorganic particles); (3) how important can be the fortnightly and seasonal variability of mercury fluxes; (4) how important seston may be for the transport of mercury (namely in toxic forms). For these purpose, mercury fractions mass balances were quantified under distinct tidal regimes (neap and spring tides) and river discharges (summer-dry and winter-rainy seasons) by means of a two-dimensional vertically integrated hydrodynamic model.

6.2 Model data set

The details of the analytical procedures and the discussion of the levels observed during the tidal cycles were presented in the chapters 3 and 4, respectively. Also in chapter 4 it was discussed the vertical representativeness of the surface samples and subsequent applicability of the hydrodynamic model. The examination of the cross-section studied in terms of the current field and turbidity pointed to a reasonable vertical homogeneity of the water column, confirmed in general by the chemical characterization of the collected samples during the tidal cycles. Only in winter neap tide an important difference was observed between surface and bottom samples, with the latter having higher SPM concentrations. The implications of this deviation from the alleged homogeneity are discussed in the present chapter.

6.3 Mass balance calculations

To estimate the fluxes of SPM, seston and mercury fractions between the lagoon and the Atlantic Ocean (and the correspondent mass balance), besides its concentrations, it is essential to know the water fluxes through the sampling cross section. In the absence of measurements of water fluxes at this location during the campaigns, a hydrodynamic numerical model (2DH) (Dias and Lopes, 2006a) was applied to compute time series of water flow through the cross section at each tidal cycle. This model has previously been applied to a study of the hydrodynamics of the Ria de Aveiro (Dias et al., 2000; 2003) and to estimate fluxes of bacterioplankton (Cunha et al., 2003) and of total suspended solids (Abrantes et al., 2006) between Ria de Aveiro and the adjacent coastal area.

The model was developed from the SIMSYS2D model (Leendertse and Gritton, 1971; Leendertse, 1987) and solves the shallow water equations. In this model, the difference equations discretized from those equations are solved by an Alternating Direction Implicit (ADI) method, using a space-staggered grid (Leendertse and Gritton, 1971). With appropriate boundary and initial conditions, this system of equations constitutes a well-posed problem whose solution describes the depth-averaged circulation in a tidal basin, and allows the determination of the water fluxes in any zone of the computational domain.

Model calibration and validation was successfully performed for Ria de Aveiro, and the results proof that the model accurately reproduce the barotropic flows in this lagoon (Dias et al., 2006a; 2006b). For the cross section sampling location (Figure 4.3), the model computes the sea surface elevation with a root-mean square error of 0.096 m, which is lower than 5% of the tidal amplitude.

Simulations were performed for each one of the sampling periods referred in section 4.2 (neap and spring tides, in winter and in summer), allowing the study of the semi-diurnal, fortnightly and seasonal variability. At the lagoon ocean boundary, the water elevation over the reference level was imposed to force the model. The sea surface elevation data used was measured at the Barra tide gauge. The wind forcing was not considered in this study, because the wind intensity during the sampling campaigns was low for the region, and in previous studies (Dias et al., 2003; Lopes and Dias, 2007) it was found that only strong and persistent winds contribute to the residual circulation in the Ria de Aveiro. For each one of the tidal cycles, time series of water flow were computed (time-step of 40 s) through the transverse cross-section of the channel that includes the sampling sections (Figure 4.3). The times of slack water were also determined from the numerical results, allowing the estimation of the time of null flux.

Instantaneous cross-sectional fluxes of SPM, seston and mercury fractions (dissolved, particulate and in seston) were estimated as the product of the appropriate concentration and the concurrent model computed water flow. Also included were the values corresponding to the time of slack water (determined from the numerical results) allowing the estimation of the time of null flux. Both water flow and most of the measured parameters are known to evolve with a nearly sinusoidal pattern in the Ria de Aveiro (Cunha et al., 2000). Using a third-degree polynomial approximation, an interpolation of the instantaneous cross-sectional fluxes was performed, in order to obtain time series of the instantaneous fluxes during each tidal cycle (Cunha et al., 2003; Abrantes et al., 2006).

Finally, mass balances of water, SPM, seston and mercury fractions (dissolved, particulate and in seston) were estimated integrating ebb and flood tide series.

6.4 Results

6.4.1 Water and SPM exchanges

The water mass exchanges between the system and the Atlantic Ocean were higher at spring tides compared to neap tides and mass balance calculations showed that the system was importing 2.0 Mm^3 and 13.5 Mm^3 during summer neap and spring tides, respectively. During winter spring tide the system exported 10.7 Mm^3, while in winter neap tide there was an import of 3.3 Mm3 (Figure 6.1). In the following representations of the exchanges it is considered that positive values represent an import to the lagoon and negative values stand for an export to the plume zone.

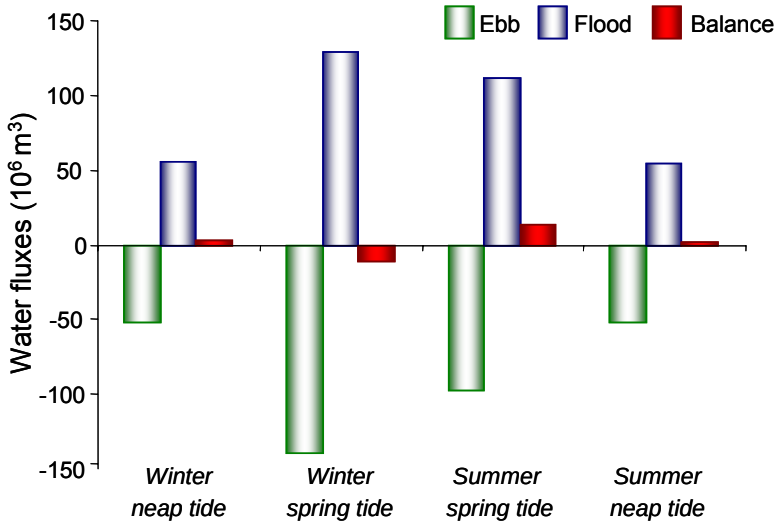


Figure 6.1 - Estimated fluxes of water (millions of m^3) through the cross section of the lagoon outer boundary and the estimated mass balance for each tidal cycle.

Regarding the fine suspended matter, seasonally the exchange of SPM between the lagoon and the sea was higher in the winter period. Yet, the estimate mass balance showed that the system imported SPM in winter neap tide (811 t) as well as in summer neap tide (257 t) (Figure 6.2). In winter spring tide the system exported 1269 t of SPM and in summer spring tide exported 409 t. During neap tides it seems that the lagoon acts as a trap for fine particles. This import of marine particles to the lagoon constitutes an important defence mechanism of the system. Particles arriving from the Ocean constitute a diluting material that mixture with the more contaminated suspended sediments coming from the contaminated areas, attenuating the total mercury burden of the SPM. This process is therefore very significant in the protection of the system, including for example for filter feeding organisms.

The process of sediment transport is extremely complex due to the interaction between numerous factors. Sediment on the bed of the lagoon is eroded and transported when the shear stress exerted on the bed by tidal, wave and freshwater action (acting either alone or together) exceeds a critical minimum value. The critical shear stress also varies according to sediment size, mineralogy and chemistry. In this manner, the combination of the seasonal and tidal forcing functions explains the higher SPM exchanges at the outer boundary cross section in winter spring tide (higher tidal range and higher river discharge), with an enhanced net export to the plume zone.

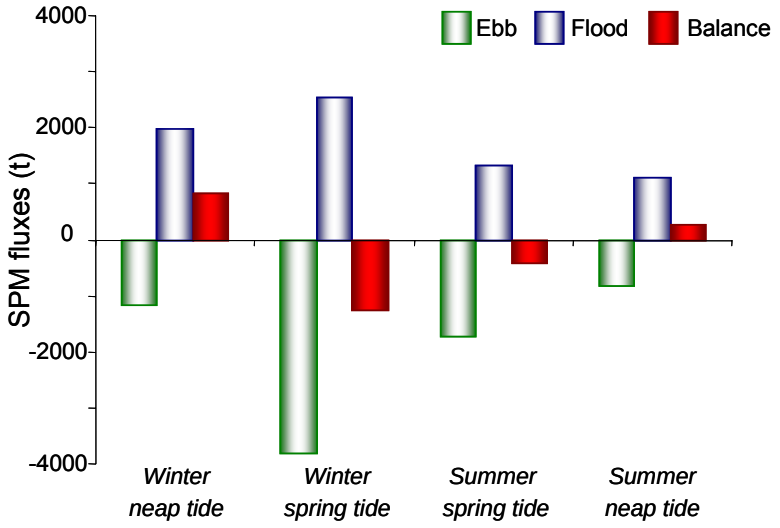


Figure 6.2 - Estimated fluxes of SPM (t) through the cross section of the lagoon outer boundary and the estimated mass balance for each tidal cycle.

In chapter 4 it was stressed that the concentration of SPM in bottom waters observed during the winter neap tidal cycle were considerably higher than the surface levels (Figure 4.4). Taking

into account that only the surface values are considered in the modelling, this higher load of SPM in the bottom waters results in an underestimation of the SPM transported during this tidal cycle and consequently will affect the result of the estimation of mercury exchanges. The importance of bedload transport is addressed in several works (FitzGerald et al., 2000; Kleinhans and Grasmeijer, 2006; van Rijn, 2007) but is almost always neglected in terms of contaminant carrier.

The seston exchanges involving the lagoon and Atlantic Ocean were more significant during winter spring tides resulting in a net export of 126 and 59 t, respectively, for seston collected with 63 µm and 200 µm mesh size plankton nets (Figure 6.3).

The seston transported during the tidal cycles was comparatively lower than exchanged SPM, reflecting the lower abundance in the water column of the coarser material retained in the nets.

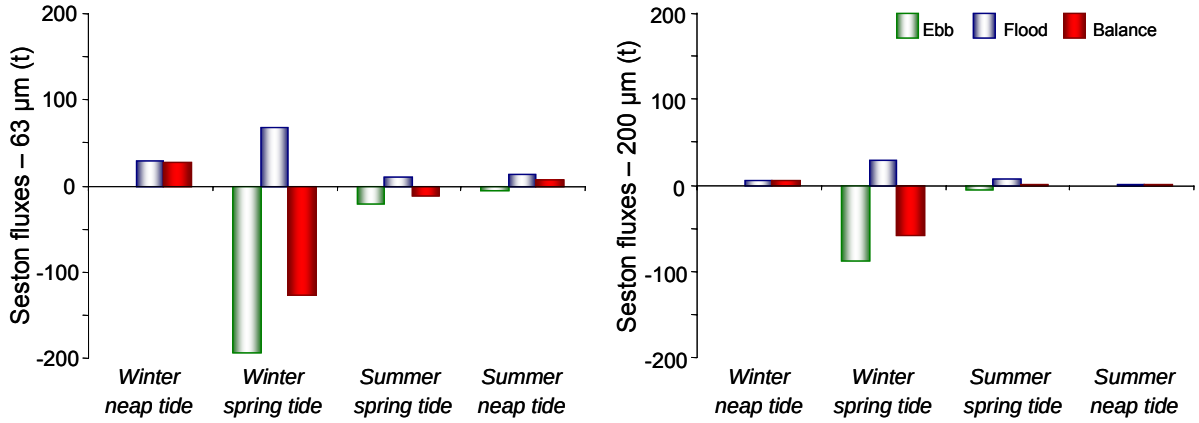


Figure 6.3 - Estimated fluxes of seston 63µm (t), seston 200µm (t) through the cross section of the lagoon outer boundary and the estimated mass balance for each tidal cycle.

The similarity between the pattern of seasonal and inter-tidal seston mass balance, with the SPM mass balance is in agreement with the suggestion of a significant contribution of detrital particles to the composition of the seston.

6.4.2 Seasonal and tidal variation of mercury exchanges

Concerning the mercury exchanges, each mercury fraction showed a distinct seasonal and inter-tidal pattern. Speaking of the total dissolved mercury fraction (Figure 6.4), the inter-tidal variations were more pronounced than seasonal variations. The exchanges in the dissolved fraction across the outer boundary were maximal at spring tides with a net import of total dissolved mercury (326 g in winter and 125 g in summer). During neap tides, mass balance calculations showed a net export of total dissolved mercury to the sea (3 and 88 g in winter and summer, respectively).

Regarding the total mercury in SPM, seasonal variations of exchanges were clearer than the fortnightly variations (Figure 6.5). Exchanges between the lagoon and the Atlantic Ocean were higher in spring tides compared with the respective neap tide and resulted in a net export of mercury (406 and 223 g in winter and summer spring tides, respectively). As previously mentioned in relation to SPM transport, and for analogous reasons, also the mercury transported associated with SPM is expected to be underestimated in the winter neap tidal cycle.

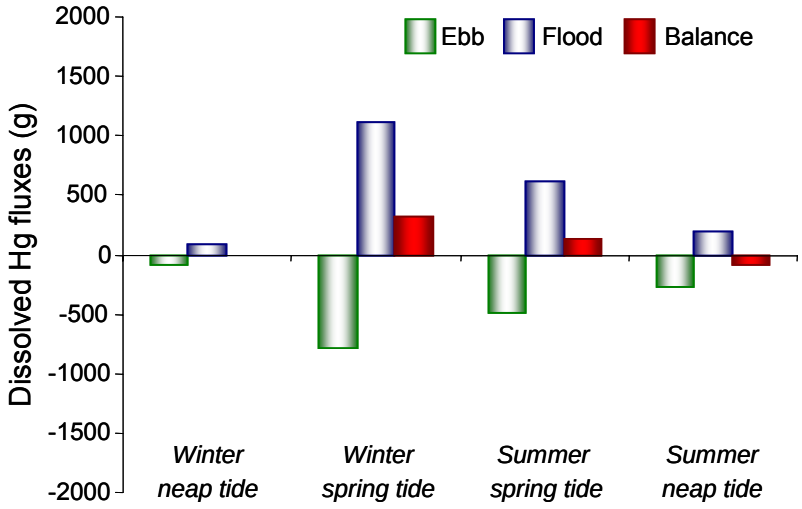


Figure 6.4 - Estimated fluxes of mercury in the dissolved fraction (g Hg) through the cross section of the lagoon outer boundary and the estimated mass balance for each tidal cycle.

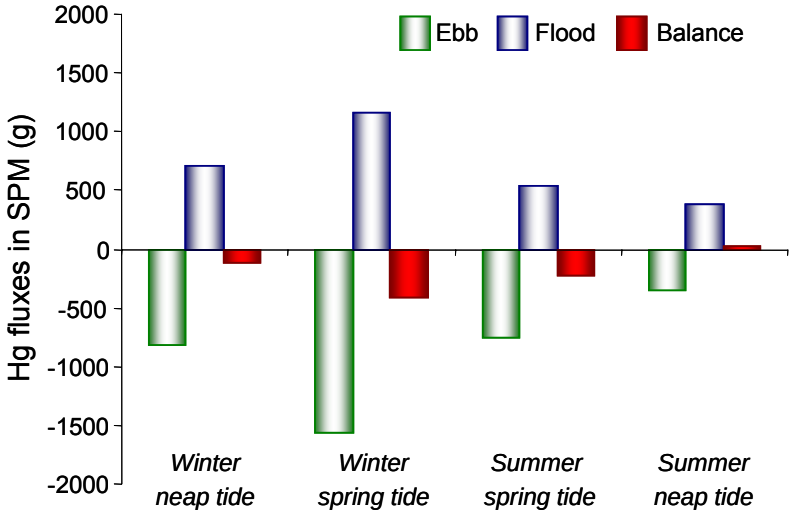


Figure 6.5 - Estimated fluxes of mercury in SPM (g Hg) through the cross section of the lagoon outer boundary and the estimated mass balance for each tidal cycle.

The dynamics of total mercury in the seston fraction (Figure 6.6) showed net export during spring tides (4.2 g in winter and 1.3 g in summer for the 63 µm mesh size net; 1.9 g in winter and

0.4 g in summer for the 200 µm mesh size net) and an import during neap tides (3.1 g in winter and 0.1 g in summer for the 63µm mesh size net; 1.4 g in winter and a insignificant amount (0.3 mg) in summer for the 200 µm mesh size net). In addition, the exchange of total mercury in seston at the outer boundary was higher during winter.

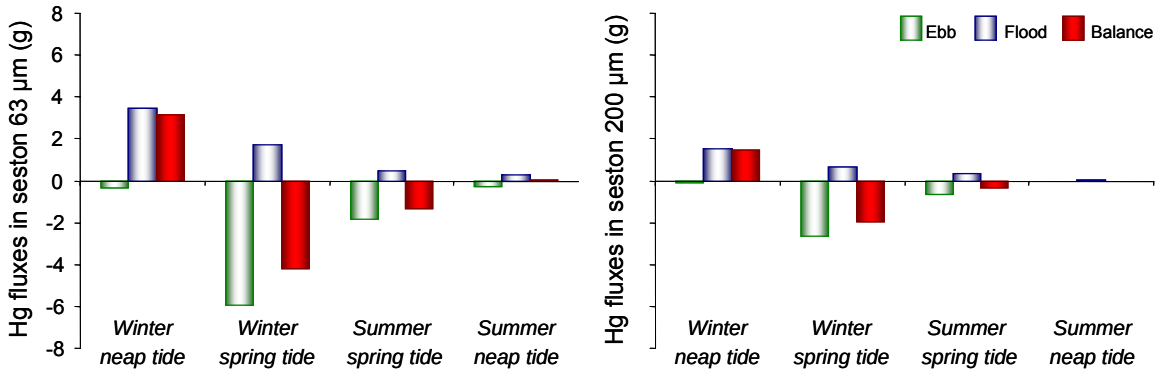


Figure 6.6 - Estimated fluxes of mercury in seston through the cross section of the lagoon outer boundary and the estimated mass balance for each tidal.

Globally, considering the relative importance of mercury transport by the different fractions, the results showed that the mercury exchanges between the lagoon and the Atlantic Ocean were higher in spring tides (Figure 6.7A).

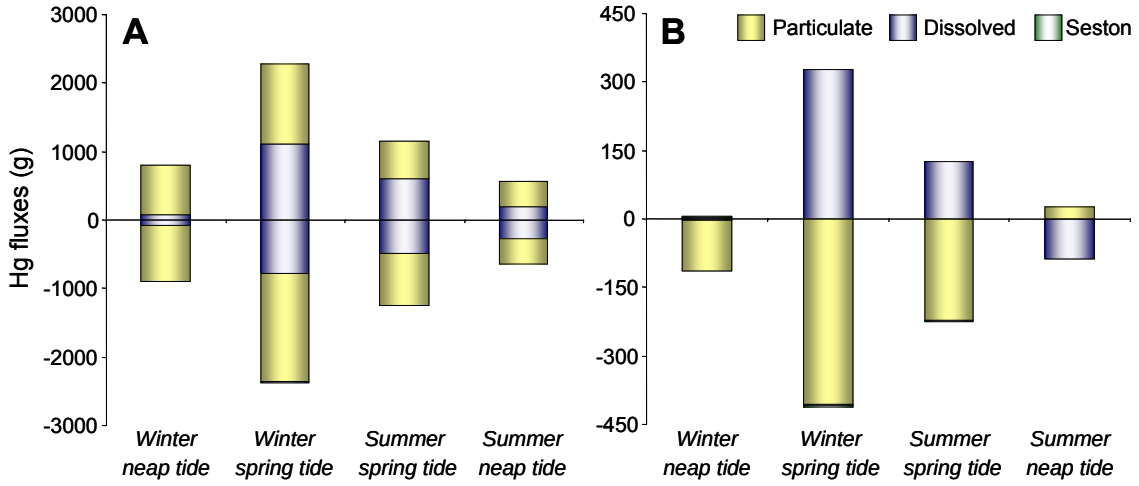


Figure 6.7 - A) Relative importance of mercury transport by the different fractions (g Hg): dissolved, particulate and in seston, and B) estimated mass balance (g Hg) for each fraction.

The relative contribution and the magnitude of mercury transported within each fraction varied both seasonally and with tidal range (fortnightly).

Considering the mass balance calculations within each fraction (Figure 6.7B) the net export of mercury observed in the four tidal cycles is mostly due to the contribution of the particulate

mercury. Nevertheless, this balance (Figure 6.7B) for each fraction also shows that the two main fractions (dissolved and particulate) had opposite behaviour in three of the four tidal cycles, with an export of metal associated with one of the fractions and an import in the other one. Only in summer neap tide was the export of mercury due to the contribution of the dissolved fraction.

The seston had an insignificant contribution to the total mercury exchanged between the lagoon and its plume zone (Figure 6.7), as a result of the presence of lithogenic components (diluting the mercury content) and mostly due to its low abundance in the water column. As a matter of fact, during flood and ebb tides, the mercury transported associated with seston was always <0.5 % of the total of mercury transported.

In addition, due to the toxicity of mercury in methylated forms, it seemed important to evaluate the contribution of organic species to the total mercury transported with seston. It was found that only 1.7-2.8% of mercury was transported in organic species during summer spring tide. Although the mechanism of methylmercury (and of other organic forms) transported from the sediments to planktonic organisms is not well known, there is evidence of the importance of the diffusive sediment flux to the bioaccumulation of methylmercury (Mason et al., 2006). Some authors, cited by Mason and others (2006), found a low flux of methylmercury from sediments under oxic conditions compared to hypoxic or anoxic conditions, suggesting that the redox state of the sediment surface may be one of the most important factors in determining the extent to which organic forms flux into the water column (Rajar et al., 1997). The low contribution of phytoplankton to seston composition and the fact that the water column is well aerated, with less bioavailable dissolved organic mercury, may contribute to the low export of organic mercury within this fraction.

Taking in account the overall balance of all fractions, during winter the system exported 0.11 kg per neap tide and 0.09 kg per spring tide, whereas in summer export was 0.06 kg per neap tide and 0.10 kg per spring tide. Although the contrasting characteristics of each survey, with tidal ranges between 1 and more than 3 m, the quantity of mercury exported are similar for the four conditions sampled. This observation is surprising given the variability in the amounts transported by each of the dissolved and particulate fractions. Considering the range over 700 tidal cycles per year, the annual export is between 42 and 77 kg (mean of 62 kg). Such extrapolation from empirical evidence over four tidal cycles to an annual of 700 tidal cycles should be faced with some cautions (Bettencourt et al., 1994). Nevertheless, the consistency of the overall balance between the surveys gives confidence to this first-order calculation. Moreover, it is in agreement

with evidences of a relatively small impact of mercury along several kilometers in the Ria de Aveiro marine coastal zone (chapter 5).

Since the daily variation of tidal amplitude occurs between consecutive tidal cycles (see deCastro et al., 2004; Figure 3) the annual mass balance calculations for water exchange at the outer boundary cross section give rise to some limitations. Nevertheless, the model is useful when estimating which mercury fraction constitutes the most important route for transport and how mercury export was controlled inter-tidally and seasonally. This kind of models is a valuable tool for the understanding of contaminant fate processes and making recovery predictions for contaminants.

It should be also emphasized that the applied modelling only comprises the pathway of outflow to the Ocean, not taking into account the exchanges with the atmosphere. Air-water exchange of mercury can be very significant in oceans margins, being a critical part of the global mercury cycle (Cossa et al., 1996; Mason and Sheu, 2002; Andersson et al., 2007; Fitzgerald et al., 2007). The exchanges depend on factors like solar radiation, wind, microbial processes and water chemistry (DOC and other dissolved constituents). As combination of this factors results in a complex temporal and geographic variability of deposition and evasion rates (Gårdfeldt et al., 2001; Andersson et al., 2007; Fitzgerald et al., 2007; O'Driscoll et al., 2007), the use of values from other systems to estimate exchanges should be highly cautious. In addition to the exchanges between air and the water column, the release of mercury from the sediment through photochemical reduction (Canário and Vale, 2004) is also worth mentioning. In a shallow system like the Ria de Aveiro lagoon, and considering that approximately 17km^2 of sediment are exposed daily to sunlight during low tide, the contribution of the evasion from the sediment should also be investigated. In the contaminated zone, the Estarreja channel margins, the high salt marsh and the intertidal mudflat in Laranjo basin constitutes a set of highly contaminated areas with a potential role in the output of mercury from the sedimentary compartment of the lagoon.

Although there is a net export from the strongly contaminated Laranjo basin (see figure 1) to the inner system (Pereira et al., 1998b; Ramalhosa et al., 2001; Monterroso et al., 2003), the alternating process of erosion and deposition associated with very shallow areas and the complex geometry of the lagoon seems to limit advective transport in the lagoon (Lopes et al., 2001). The predominance of strong associations of mercury with particles in the estuarine environments (Le Roux et al., 2001) is also importance for the hindrance of mercury dispersion through dissolved forms.

The residence time in the contaminated area (about 2 weeks and more; Dias et al., 2001), appears to play a crucial role in the retention of mercury, by hindering the export to the main body of the lagoon. This residence time was obtained through particle tracking under passive dispersion and hence sub estimates the panoply of the processes promoting the recycling and trapping of particles in estuarine environments. In fact, the seaward movement of eroded sediments can also be delayed by flood dominance in Laranjo basin (Lopes et al., 2001), with stronger current speeds resulting in higher erosion during the flood tide. Additionally, most of the water that leaves the basin during ebb tide is pushed back when the tide reverts. In the mercury exchanges between Laranjo Basin and the main body of the lagoon, it was observed that almost 70% of the particulate mercury that leaves Laranjo during ebb tide, is pushed back in during the flood (Ramalhosa, 2002).

The existence of a complex cycle of resuspension/deposition processes for sediments resuspended in Laranjo basin can also cause difficulties for the net transport of sediments seaward and should be further investigated. All the factors related with this internal cycling contribute to an effective retention of mercury in the contaminated inner basin, which protects the main body of the lagoon and the coastal Atlantic zone from severe contamination, but delays the recovery of the impacted inner zone. In fact, considering the estimated quantity of mercury exported per year to the Ocean, the recovery of the lagoon from contamination of deep sediments is likely to remain a long-term issue (300 years for 50% reduction). Nevertheless it must be stressed the importance of high flow periods that show a prevalence of Hg transport (Coquery et al., 1997; Schäfer et al., 2006).

7. Synthesis and Outlook

In its mysterious past it [the sea] encompasses all the dim origins of life and receives in the end, after, it may be, many transmutations, the dead husks of that same life. For all at last return to the sea — to Oceanus, the ocean river, like the everflowing stream of time, the beginning and the end.~ Rachel Carson (1907-1964).

The high mercury concentrations found in many aquatic systems, as a result of past anthropogenic emissions, should make the assessment of both its transport and dispersion to nearby coastal zones a priority. Studies focused on contaminant fluxes to coastal zones can be useful tools for impact assessment to shelf seas, providing complementary information to the monitoring of concentration levels. Considering that transport and dispersion have a crucial influence on the time and space distribution of contaminants, hydrodynamic models are very useful tools in analysing cycling processes. Furthermore, calculations of mass-balance help to assess the relative importance of sources and sinks, and provide a good insight into the problem, being indispensable to evaluate the evolution trend and recovery time of contaminated coastal systems, and consequently for the good management of the resources. The evaluation of the future development of contaminated aquatic environments should be regarded as an invaluable tool for decision making on remediation efforts.

Considering that the Water Framework Directive requires a coherent and comprehensive assessment of water bodies' ecological and chemical status (related to the level of concentration of priority substances in the water column, sediments and biota), the evaluation of the mercury distribution in the coastal waters of the Ria de Aveiro can be considered of great value and useful for the optimization of monitoring programs. Data of estuarine inputs constitutes also useful tools for management, trend analysis and the identification of pressures. In addition, this knowledge about the environment of the OSPAR maritime area is indispensable to update and refine assessment criteria.

In the Ria de Aveiro, high levels of mercury are found inside some areas of the lagoon, which, together with an hydrodynamic circulation essentially dominated by tidal forcing, results in a daily mercury transport to the coastal zone during the exchange of the water mass.

Considering the distribution of mercury during the tidal cycles of different river discharge and tidal ranges, the maximum total dissolved mercury at the outer boundary was one order of magnitude lower than the concentrations of total dissolved mercury found in previous studies at inner areas of the lagoon (e.g, 275 ng L^{-1}, Coelho et al., 2005; Table 2). In addition, 96% of the determinations were <15 ng L^{-1} and 80% <10 ng L^{-1}, demonstrating that higher values were not frequent. These total dissolved concentrations were quite similar to those found in the coastal zone. In fact, the overall mean concentration at the outer boundary (6.6 ± 0.7 ng L^{-1}, 95% confidence, n=143) was not significantly different (Mann-Whitney Rank Sum Test) from the overall average concentration at the system nearshore (7.3 ± 1.1 ng L^{-1}, 95% confidence, n=95).

The suspended particulate matter is the main carrier of mercury in the inlet, with the higher concentrations of mercury in SPM observed during the winter neap tidal cycle (levels up to 1.0 µg g^{-1}). The contribution of particulate Hg to the total mercury is only below 50% when the SPM concentration is low (<30 mg L^{-1}) and increases non-linearly with increasing SPM concentrations, suggesting that the relative abundance of particulate Hg appears to be controlled by the SPM concentration to a significant extent. For high SPM concentrations, the mercury exists predominantly (80 - 90%) in the particulate fraction. In addition, the composition of both the suspended particulate matter and the seston varied with the different river discharge and tidal ranges, influencing the mercury content of particles and the partitioning between dissolved and particulate fractions. All data considered, mercury distribution and reactivity were found to be a result of a complex interaction of several factors, with an important role played by tidal currents, wave energy and wind.

Considering the observed variability of the seston composition related with tidal energy, it should be noted that for environmental research focusing on plankton bioaccumulation, trophic transfer and transport of contaminants, it will hence be essential on one hand, to minimize sampling bias by adjusting sample collections to slack periods (when resuspension is minimal and the biogenic fraction of seston reach maximum levels). On the other hand is important to study the partition between biogenic and lithogenic fractions in samples, to improve the accuracy and validity of the findings.

The highest concentrations of mercury in seston were observed during winter (maximum of 0.41 µg g^{-1}) and in general concentrations increased during the ebb tide. In the winter spring tide survey, the maximum mercury concentrations observed at the end of the ebb tide were 30 times higher than minima levels at the end of the flood tide, evidencing the important source of mercury located upstream. Concentrations in seston collected in the 200 µm net were generally higher, denoting a bioaccumulation of mercury.

Considering the mercury transport, the highest mercury exchanges during flood and ebb were observed in winter spring tide conditions. Results of mercury fluxes estimates showed that, compared to the total dissolved mercury and total mercury in seston, the particulate fraction plays an important role in the transport of mercury. The seston had an insignificant contribution (always <0.5%) to the total mercury exchanged between the lagoon and its plume zone, as a result of the presence of lithogenic components (diluting the mercury content) and mostly due to its low abundance in the water column. During spring tides, in summer, about 2% of the mercury

transported in seston was present in an organic form. The mass balance for the mercury fractions revealed that the mercury exports to the Atlantic Ocean varied with season and tidal regime, mainly in terms of the relative importance of the dissolved and particulates fractions. Thus, the variability in particle-water interactions is particularly important for the establishment of long term metal budgets and to assess the ecological risks due to the local oceanic budget of mercury. Bearing in mind that mercury exchanges within the dissolved and particulate fractions had an opposite behaviour in most of the tidal cycles, the end result will depend on the magnitude of the role of each fraction, which reinforces the importance of the study of the partitioning in the transport. In addition, the variability of mercury partitioning in the export of the metal to the near shore means that organisms will be preferentially and differently exposed to these fractions at different times of the year. Since the target species may differ from feeding activity (*e.g.* filter-feeders, deposit feeders or scavengers) and rate of exposure (*e.g.* benthic or pelagic organisms), and the bioavailability may be affected by geochemical effects (Luoma and Rainbow, 2005), the prevalence of mercury bioaccumulation in these species may also vary. Namely, several macrobenthic species with economical interest (*e.g. Carcinus maenas, Scrobicularia plana, Cerastoderma edule*) have a planktonic phase during their life cycle.

An approximate range of values for the annual mass balance between 42 and 77 kg show that the export of dissolved and particulate mercury has little impact on the nearshore region of the Atlantic Ocean. In fact, considering the estimated quantity of mercury exported per year to the Ocean, the recovery of the lagoon from contamination of deep sediments is likely to remain a long-term issue (300 years for 50% reduction).

The representativeness of the obtained annual budget is an open question and the variability from year to year should also be taken into consideration and investigated. As an example, the effects of high energy phenomena like floods and storms, far from being negligible, are known to be major routes of transport.

The budget obtained of mercury exchanges between the Ria de Aveiro and the Ocean are comparable with the results found in previous studies for the exchange between the contaminated area and the lagoon main body. These observations are also in agreement with the knowledge of the hydrodynamic of the lagoon, where it has been documented that a very low residence time for the main channels, results in a rapid flushing of mercury that reaches zones of no return.

The limited transport of mercury from the contaminated area is consistent with the decrease of mercury concentrations in the transition between the Laranjo Basin and the main body of the

lagoon. In fact, it seems that mercury is efficiently retained in the contaminated area (Laranjo basin), even though previous studies reported an export of mercury to the main body of the lagoon. The immobilization of metals aided by the chemistry of sulphur species in sediments of the Laranjo basin has already been reported for mercury (Ramalhosa et al., 2005) and also for other metals (Monterroso et al., 2007). Nevertheless, until now, no evaluation had been done taking into account the variability of tidal range and river discharge. Although most of the mercury in the mentioned inner basin is buried in sediments at 30-40 cm deep, high mercury concentrations are still observed in the surface sediments. In spite of this, the results of the present study show that the dispersion of mercury from the inner contaminated basin is not as quick as it would be expected, with a crucial role played by the residence time in this zone (about 2 weeks according to Dias et al., 2001 and more than 20 days as suggested by Trancoso et al., 2005). Most of the water that leaves the basin during ebb tide is pushed back when the tide reverts. The tidal asymmetry reported for the shallow areas and the end of the channels results in higher erosion during floods with recycling of sediments to upstream areas. Together with internal cycling through cycles of resuspension/deposition, these factors contribute to an effective retention of mercury in the contaminated inner basin (a turbidity zone as identified in the channel connecting Laranjo to the main body of the lagoon; Lopes et al., 2006b), which protects the main body of the lagoon and the coastal Atlantic zone from severe contamination, but delays the recovery of the impacted inner zone.

The dimension of the residence time in Laranjo basin makes this area advantageous for phytoplankton growth because it resides long enough to use the dissolved nutrients (Trancoso et al., 2005). The levels of mercury in the water column of the basin constitute therefore a risk for the contamination of the base of the food chain and subsequent biomagnification in the Ria.

It should be recognized that it is the high contamination of the Laranjo basin (result of its efficient retention capacity) that allows the sustainability and productivity of the Ria de Aveiro. Considering management interests of the Ria, the results of the present study reinforce the vital significance of leaving the contaminated sediments undisturbed and that uncontrolled dredging is highly unwise in the contaminated area. In addition, facing the persistence of the contamination, conditions for methylation should be prevented. This is a very hard task, which is mainly attainable by improvement and maintenance of the water quality in the Ria de Aveiro.

The results of the mercury mass balance are also in agreement with the limited impact observed on the nearby marine area on a scale of several kilometres, especially when compared

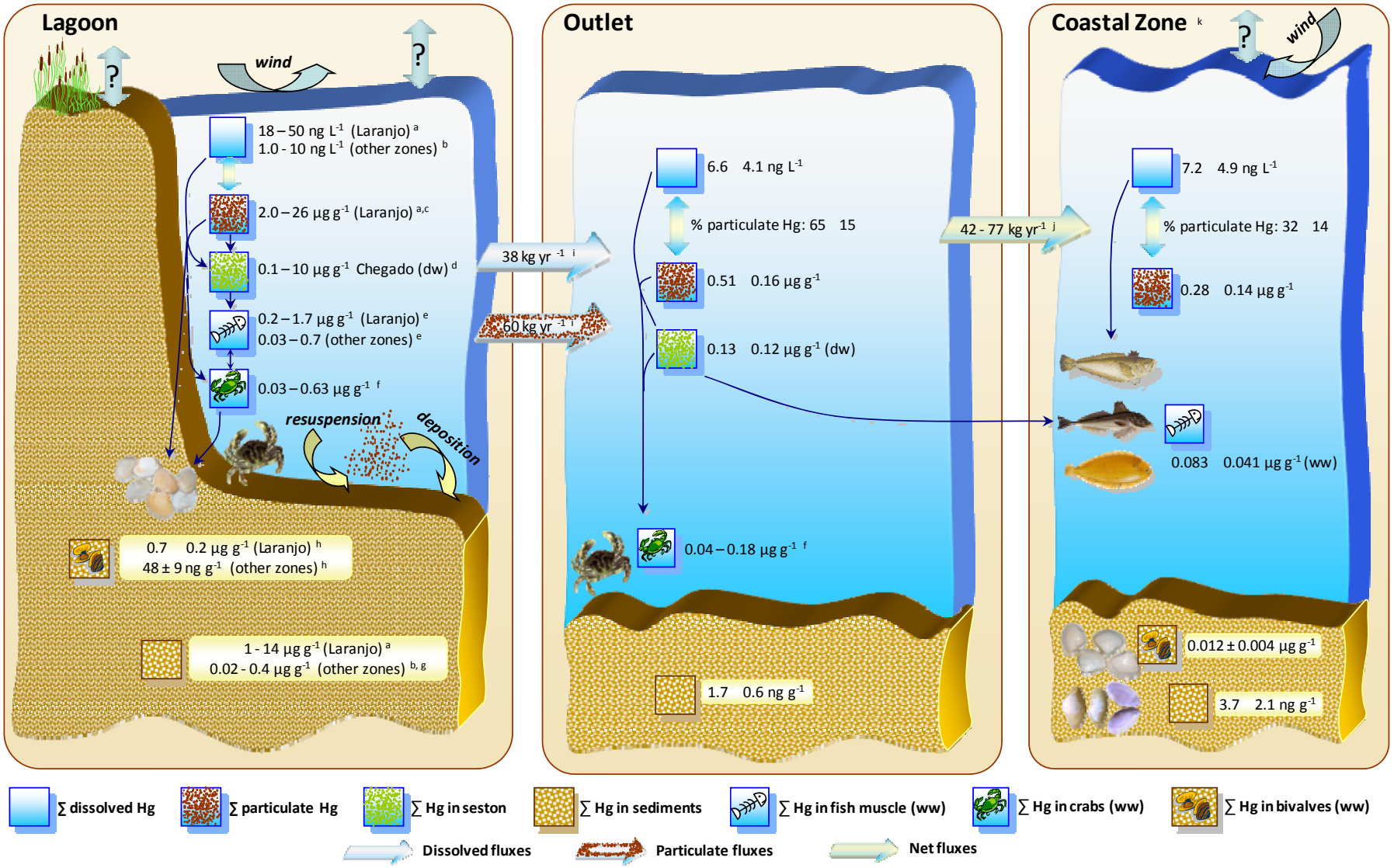
with the levels found inside the lagoon. In the adjacent Atlantic coastal area, mercury related determinations showed no seasonal pattern in all the compartments studied.

Although organisms of the coastal zone exhibited some bioaccumulation of mercury, levels are below regulatory guidelines with no evidence of mercury pollution posing a threat to human health in the marine coastal zone. In spite of the mentioned limited impact, some indicators were found that are typical of impacted zones. Levels rise above background reference concentrations only in the water column, particularly in suspended particulate matter, due to the high affinity of mercury for particulate matter. The long range transport of these small dimension particles (together with the low efficiency of local sandy sediments to trap mercury) will play an important role in the dispersion of mercury transported in suspended particulate matter over a wider/broaden area and reducing the impact in the nearby marine coastal zone.

The Figure 7.1 represents a simplified diagram with some contributions of the present study to the biogeochemical cycle of mercury in the Ria de Aveiro and its coastal zone. It summarizes the present knowledge of mercury distribution in several environmental compartments obtained from several studies, stressing the importance of some processes that influence the mercury cycling in the system and highlighting the bioaccumulation of mercury in biota. It also indicates the missing information concerning the importance of contribution on air-water exchanges and sediment-air exchanges in the cycling of mercury in the Ria de Aveiro.

During the course of such an investigation, many questions arise with unquestionable importance. Some of them are difficult to answer without further investigations and they constitute a valuable source of issues for further studies:

- Influence of climate change scenarios (changes in fluvial input and of tidal incursion due to sea level rise) in the mercury cycling in the lagoon and exchanges with the Ocean.
- Relative contribution of high-energy phenomena to the transport of mercury to the Atlantic Ocean.
- Further investigations of mercury speciation in the water column, namely through studies of colloidal forms and mercury hidrophobicity.
- Study of contributions of air-water (gaseous and through aerosols) and sediment-air exchanges to the cycling of mercury in the Ria.



[a] Ramalhosa et al., 2006b; [b] Coelho et al., 2005; [c] Ramalhosa et al., 2006a; [d] Total Hg in seston (dw) in 63 and 200 µm net (Monterroso et al., 2003); [e] Abreu et al., 200; [f] Total Hg (ww) in muscle and hepatopancreas of *Carcinus maenas* (Pereira et al., 2006); [g] IDAD, 2001 ; [h] Total Hg (ww) in 2.5 years old *Scrobicularia plana* (Coelho et al., 2006); [i] Ramalhosa, 2002; [j] Pato et al., in press; [k] Pato et al., 2008

Figure 7.1 - Generic model of distribution of total (∑) mercury concentrations in the Ria de Aveiro lagoon and the adjacent coastal zone (ranges and mean ± standard deviation).

In addition to these topics it should be recalled that a study of mercury distribution over a wider salinity range would be very useful to clarify the factors governing mercury behaviour during the estuarine transit in the Ria de Aveiro.

With several global strategies intending to reduce the uses, emissions and supply of mercury, the transport and fate of this contaminant following re-mobilization will remain a key issue of environmental concern. Considering that factors promoting sediment resuspension were greatly implied in the results of the amounts exchanged in the Ria de Aveiro, high energy phenomena like floods and storms are likely to contribute to an enhanced export of mercury from the contaminated area, both through the resuspension of mercury enriched particles and also by the addition to the water column of mercury enriched pore waters. In this context, climate change scenarios with increasing frequency and magnitude of storms, flood risks and sea level rise are of major concern.

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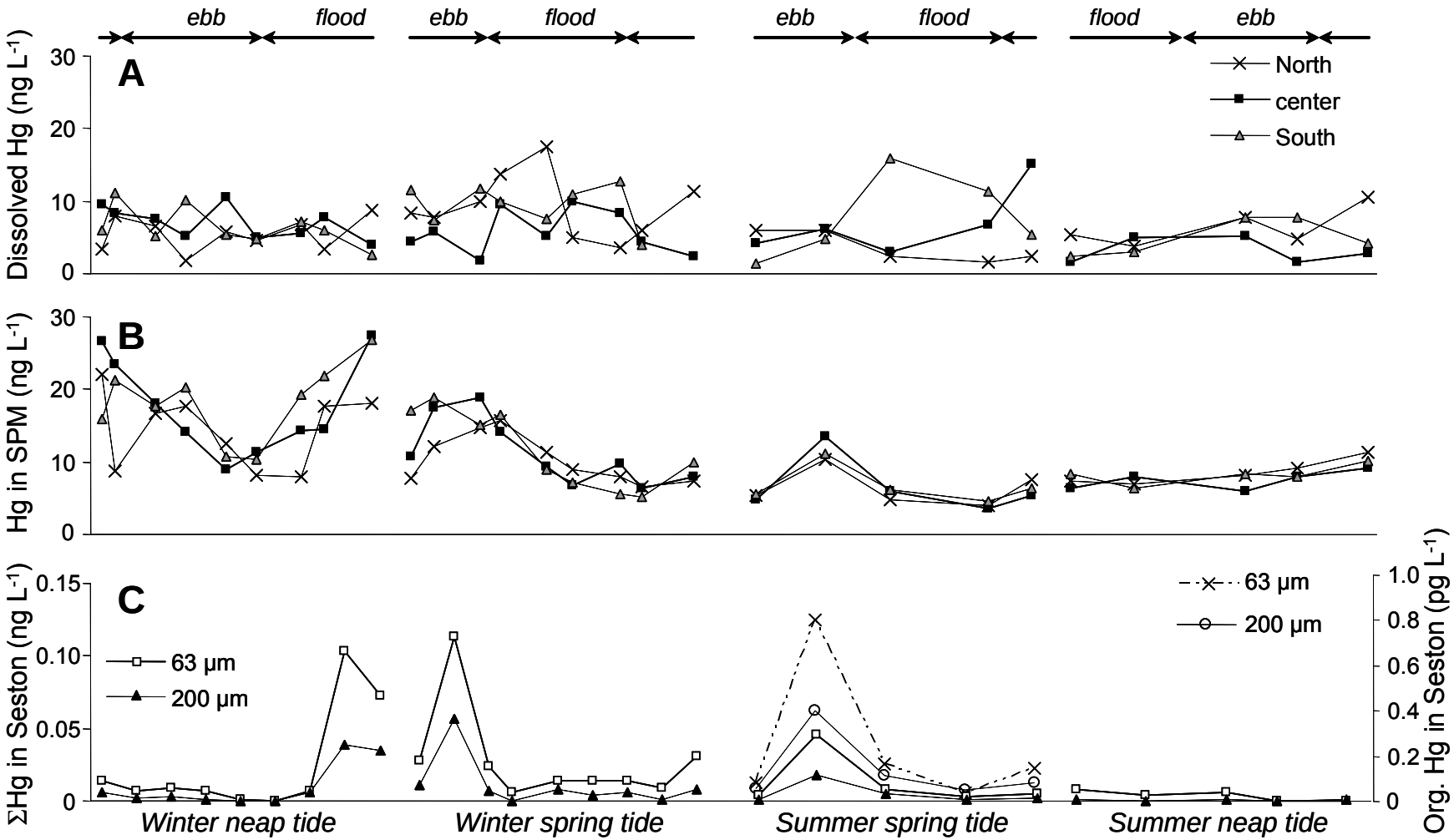
ANNEX I

Concentrations used in the numerical modelling



Seasonal and tidal variation of mercury (ng Hg L^{-1}) fractions (dissolved, particulate and in seston) during tidal cycles